A Dissertation on

A STUDY ON HAEMATOLOGICAL CHANGES FOLLOWING FIRST LINE HIGHLY ACTIVE ANTI RETROVIRAL THERAPY IN ADULT HIV -1 INFECTED PATIENTS.

Dissertation Submitted to

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for the award of the degree of

M.D. GENERAL MEDICINE

BRANCH – I

COIMBATORE MEDICAL COLLEGE,

COIMBATORE

APRIL 2015

CERTIFICATE

Certified that this is the bonafide dissertation titled **"A study on Haematological Changes following first Line HAART in Adult HIV -1 Infected Patients"** done by **Dr. Ramalingam P K** and submitted in partial fulfilment of the requirements for the Degree of **M.D General Medicine**, Branch I of **The Tamilnadu DR. M.G.R. Medical University Chennai.**

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DECLARATION

I solemnly declare that the dissertation titled **"A study on Haematological Changes following first Line HAART in Adult HIV -1 Infected Patients"** was done by me from AUGUST 2013 to JULY 2014 under the guidance and supervision of Professor **Dr.KUMAR NATARAJAN M.D.,**

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A study on
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HAART (Highly Active Anti retroviral Therapy)
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LIST OF ABBREVIATIONS USED

ABC	-	Abacavir
AFB	-	Acid-Fast Bacilli
AIDS	-	Acquired Immunodeficiency Syndrome
ALT	-	Alanine amino transferase
ART	-	Antiretroviral Therapy
ARV	-	Antiretroviral (drug)
AST	-	Aspartate Aminotransferase
ATV	-	Atazanavir
AZT	-	Zidovudine (also known as ZDV)
CD4	-	T-lymphocyte
d4T	-	Stavudine
ddI	-	Didanosine
EFV	-	Efavirenz
FBC	-	Full Blood Count
FDC	-	Fixed-Dose Combination
GI	-	Gastrointestinal
Hb	-	Haemoglobin
HIV	-	Human Immunodeficiency Virus
HAART	-	Highly active antiretroviral therapy
HIVDR	-	HIV Drug Resistance
IDV	-	Indinavir
IRS	-	Immune Reconstitution Syndrome
NACO	-	National AIDS Control Organisation

NFV	-	Nelfinavir
NNRTI	-	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	-	Nucleoside Analogue Reverse Transcriptase Inhibitor
NVP	-	Nevirapine
PCP	-	Pneumocystis Pneumonia
PCR	-	Polymerase Chain Reaction
PGL	-	Persistent Generalized lymphadenopathy
PI	-	Protease Inhibitor
PLHA	-	People Living With HIV/AIDS
PMTCT	-	Prevention of Mother-to-Child Transmission (of HIV)
RDA	-	Recommended Daily Allowances
RNA	-	Ribonucleic Acid
SQV	-	Saquinavir
TB	-	Tuberculosis
TDF	-	Tenofovir Disoproxil Fumarate
TLC	-	Total lymphocyte Count
WHO	-	World Health Organization
NNA	-	Normocytic Normochromic Anaemia
MHA	-	Microcytic Hypochromic Anaemia
NHA	-	Normocytic Hypochrromic Anaemia
MHA	-	Macrocytic Hypochromic Anaemia

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ABSTRACT

Background and Objectives

The first six months after the initiation of HAART is critical. Although clinical and immunological improvement is expected, It is not always apparent and the drugs may have side-effects. Anaemia and neutropenia ,Sudden and acute bone marrow suppression can occur within the first weeks of therapy or present as a slow onset of progressive anaemia over months. Most of the toxicity/side-effects can be adequately co-managed with efficient clinical monitoring at all levels of the health care system.

Objectives.

1.To study the Haematological Changes in Adult HIV Patients following first line HAART initiation.

2. To study if there are any contributory baseline factors for the Haematological changes Post HAART.

Data Collection and the Source.

HIV Patients admitted to Medical Units with Haematological changes after initiation of first line HAART during the study period. HIV Patients Reviewed at the ART Centre at CMCH with Haematological changes soon(day 15,1 month,6 month) after the initiation of first line HAART during the study period.

Data collection Methods:

All Adult HIV patients between the time period of Aug 2013 till Aug 2014 Who developed Haematological Changes Post First Line HAART initiation was included in the study.

Sampling Method.

Prospective Cohort Study with Consecutive Sampling

Sample Size : 52 Patients.

Case Definitions.

Defined as per the National Aids Control Organisation (NACO) HIV/ART guidelines (First line HAART, Anaemia < 10 gms %).

Results.

The age in the study group ranged from 18-56 with 85 % in the reproductive age group. The percentage of severe anaemia <7 were 46.2 % in the study population. The most common type of Anaemia in the study was Microcytic Hypo chromic Anaemia found in 41% of population. Besides Anaemia there were 13% incidence of Pancytopenia and the incidence of Thrombocytopenia was 42 %. There was no significant correlation between the occurrence of Pancytopenia and the baseline CD4 counts. The second most common pattern was that of Macrocytic Hypo chromic Anaemia in about 23 % of patients. Among the clinical symptoms Fatigue and dyspnoea were found in 34 % . There was a significant rise in haemoglobin and

Hematocrit after the initial drop due to AZT and it was observed especially in those patients who developed anaemia within 90 days of ART. The bone marrow findings did not bring about any significant correlation .

Conclusion;

There is a significant rise in Haemoglobin and the Hematocrit within a median time duration of 6 months after the change from AZT based regimen.

The Baseline CD4 counts, Age and Sex did not significantly contribute to the development of Cytopenias in this study but the AZT based regimen had a significant impact contributing to all the cases in the study population.

Keywords: Human Immuno deficiency Virus, Highly active antiretroviral therapy, Cytopenias.

INTRODUCTION

Hematologic aberrations are widely seen in Human immune deficiency virus-1 (HIV -1) infected patients and they assume significant clinical importance in the treatment of a person living with HIV/AIDS (PLHIV).

HIV causes various disturbances of immune function and hematologic defects characterised by Cytopenias of individual as well as multilineages of the hematopoietic system. Varying degrees of myelosuppression are seen as a part of the dose limiting toxicities of various drugs used in the treatment of HIV and other opportunistic infections.

Though the drugs remain the primary cause of hematologic abnormalities there are a wide range of causes linked directly to the infection of the blood lymphocytes, monocyte and macrophages by the HIV-1 infection. These effects have been produced by the marked effects on the cytokine production which in turn acts on the hematopoietic system producing a range of effects like ineffective haematopoiesis and rapid apoptosis.

Besides the direct role played by HIV-1 ,other opportunistic infections, malignancies, and drugs also do play a significant role in the higher incidence of Cytopenias among HIV-1 infected patients . The steps taken to correct these defects like inhibition of HIV replication by the use of anti retroviral drugs (ART) ,treatment of opportunistic infections and malignancies, substitution of Myelosuppressive medications, treatment of the associated nutritional or other deficiencies and augmentation of the hematopoietic progenitor cells with recombinant factors, in turn facilitates proliferation , differentiation and maturation of all the affected blood cells .

The specific Cytopenias seen in asymptomatic HIV -1 infected individuals are anaemia occurring in up to 17 % of individuals ,neutropenia in up to 8 % of individuals, and thrombocytopenia in 13 % of individuals and these vary considerably with advancing HIV infection. These Cytopenias contribute considerably to the morbidity and the mortality associated with the Acquired immune deficiency syndrome (AIDS) as the dose reductions and interruptions of the offending drugs often lead to the emergence of drug resistance and the progression of the disease .

Cytopenias are an independent prognostic marker in HIV progression and death apart from the CD4, Viral load. ART is helpful in combating Neoplasm, Recurrent OI's, and thereby improving quality of life. Among the ART, there is an increased incidence of anaemia in the ZDV based regimen, which suppresses the bone marrow by a variety of mechanisms.

This being the one of the leading cause of substitution in the first 6 months of starting therapy ,and a detailed study of this event will be helpful in the better management of anaemia and other Cytopenias with the ZDV based ART regimens.

In the study called the Multicenter AIDS cohort study where the incidence of anaemia was calculated among HAART naive and on HAART HIV-1 infected patients it was observed that about 3.2 % of patients were anaemic and they had higher CD4 counts (> 700) and in those persons who had low CD4 counts(<250) the incidence was found to be about 20.9 %.

It has also been observed that anaemia and Granulocytopenia occurs in a severity which parallels the course of the disease it is not the same with thrombocytopenia as it can occur independently at all stages of HIV-1 infection.

Unexplained anaemia (<8 g/dl), neutropenia (<0.5 x 10^9/litre) and or chronic thrombocytopenia (<50000), have been described under the stage 3 of the WHO staging for adults and adolescents and various studies have shown the improvement of these parameters with increase in CD4 counts.

AIM OF THE STUDY

The aim of the study is to Analyse the Haematological changes after the Initiation of First line HAART in adult HIV one patient.

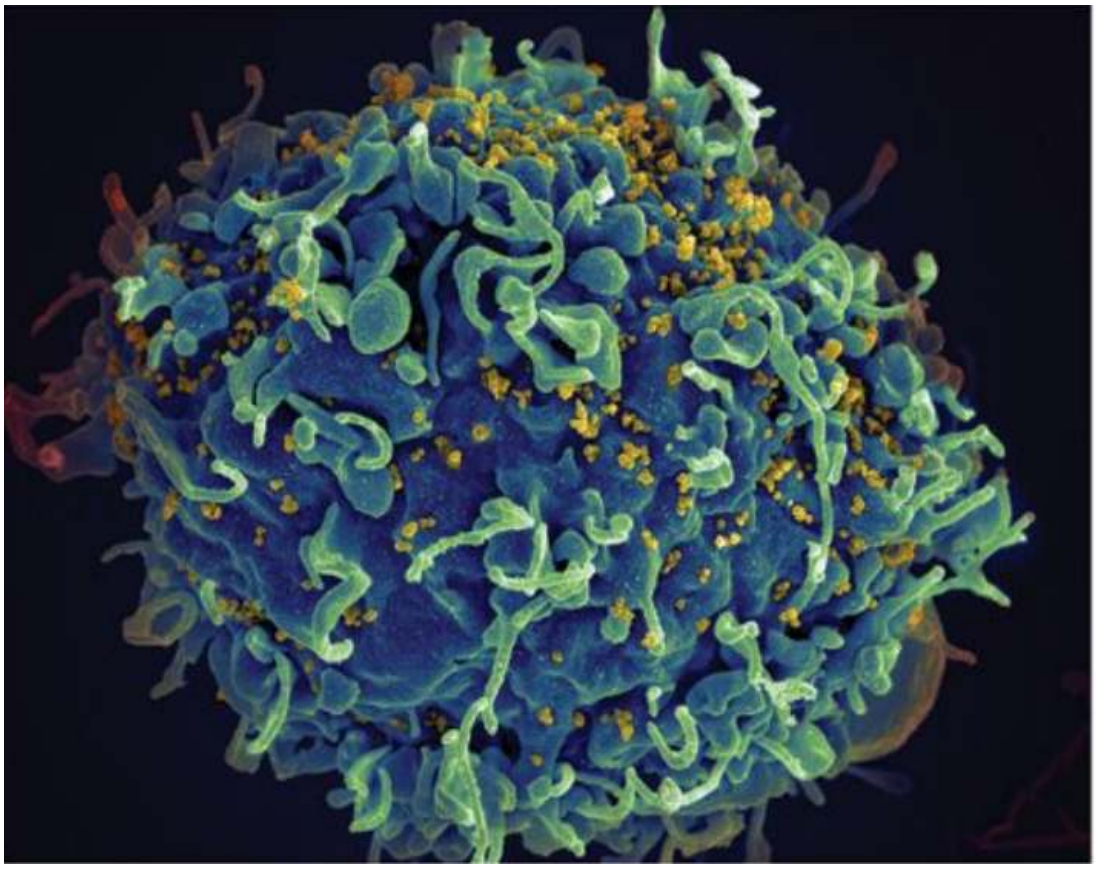
Objective Of The Study

1.To study the Haematological Changes in Adult HIV Patients following first line HAART initiation.

2. To study if there are any contributory baseline factors for the Haematological changes Post HAART.

REVIEW OF LITERATURE

The syndrome of AIDS is caused by Human immune deficiency virus which was first reported from the United states in the year 1981 when it was a chance observation seen in a few patients who had Pneumocystis jiroveci (previously called P.Carnii) infection and also had Kaposi's sarcoma. These patients were otherwise healthy and were homosexual men.



Soon the disease syndrome was documented among people who were intravenous drug abusers, patients who received repeated blood transfusions and in haemophiliacs, in female sexual partners of men who had the AIDS symptomatology and also in children born to mothers with the AIDS complex. It was in 1983 that the link was established between the HIV virus and AIDS when the virus was isolated from a person with persistent generalised lymphadenopathy [1] . Subsequently in 1985 a diagnostic antibody based testing called the Enzyme linked immunosorbant assay(ELISA) was developed as a screening test with almost 99 % sensitivity and since then has become the important diagnosing tool to detect HIV .

The innate immune systems in the body produces the defensive white blood cells and antibodies to attack the various infective organisms like the bacteria ,viruses, fungi and parasites that enters the body .These defensive cells are the T-cell lymphocytes, B lymphocytes and the natural killer cells. Once the infective organisms namely the HIV enters the body and when it starts attacking the T lymphocytes it cause the gradual depletion of the CD4 cells .These CD4 Cells are the primary defences against the viruses and other capsulated organisms that enter the body [2] .Once the depletion starts the immune system stands weakened and the body stands to be invaded by various diseases caused by the so

called opportunistic infections and also by various malignancies. The most common opportunistic infections are Tuberculosis, cryptococcosis, candidiasisis, *Pneumocystis jiroveci* pneumonia , Toxoplasmosis, Cryptosporidiosis, and Herpes zoster. The common malignancies are Kaposi's sarcoma, primary CNS lymphoma of the brain and carcinoma of the cervix to name a few. These various manifestations needs to be controlled effectively and there are various measures like the Highly active retroviral therapy (HAART) and the treatment for specific opportunistic infections which needs to be started early during the course of illness to control the disease progression and prolong the survival of the patient [3] .

The Etiologic Agent .

The etiologic agent is the Human immune deficiency virus (HIV) which is part of the retroviridae family and it belongs to the subfamily of lentivirdae.

There are four virus part of the retroviridae group and they are Human T Cell lymphocytotropic virus (HTLV I and II) which are mainly transforming retrovirus and HIV -1 & 2 which cause mainly cytopathic effects (killing the cells)in due course . HIV -1 is the most common cause of disease all throughout the world and HIV 2 is predominantly found in the African subcontinent (mostly in west African patients) [4].

There are several groups to the viruses and the types associated with HIV 1 are (M,N,O,P) and the subtypes associated with HIV 2 are (A-G). The origin of these virus have been traced to a non human primate reservoirs. The HIV -1 has been traced to chimpanzees and gorillas and the HIV-2 has been traced to sooty mangabeys[5,6,7.]. The pandemic around the world is caused mainly by HIV-1 M subgroup virus though subgroup O and HIV-2 has also been documented in many more localised epidemics in the developed world.

The group M is called (major), and is responsible for the infections in the American and European continents and the group O is called the (outliers),a somewhat rare forms found in Cameroon and Gabon and another group N so called because it is novel or it is also called as non-M-non- o which is also seen around Cameroon. There are several subtypes or clades to these groups and there are also certain forms called the circulating recombinant forms which are expanding now greatly across the globe . These CRFs are formed by two subtypes that infect the same individuals which then recombine that creates a virus with a selective advantage[8].

The geographic distribution of the HIV -1,2 groups ,subgroups ,and the various recombinant forms are demonstrated in the figure 1.The Subtype C accounts for almost approximately half of all infections across the globe .

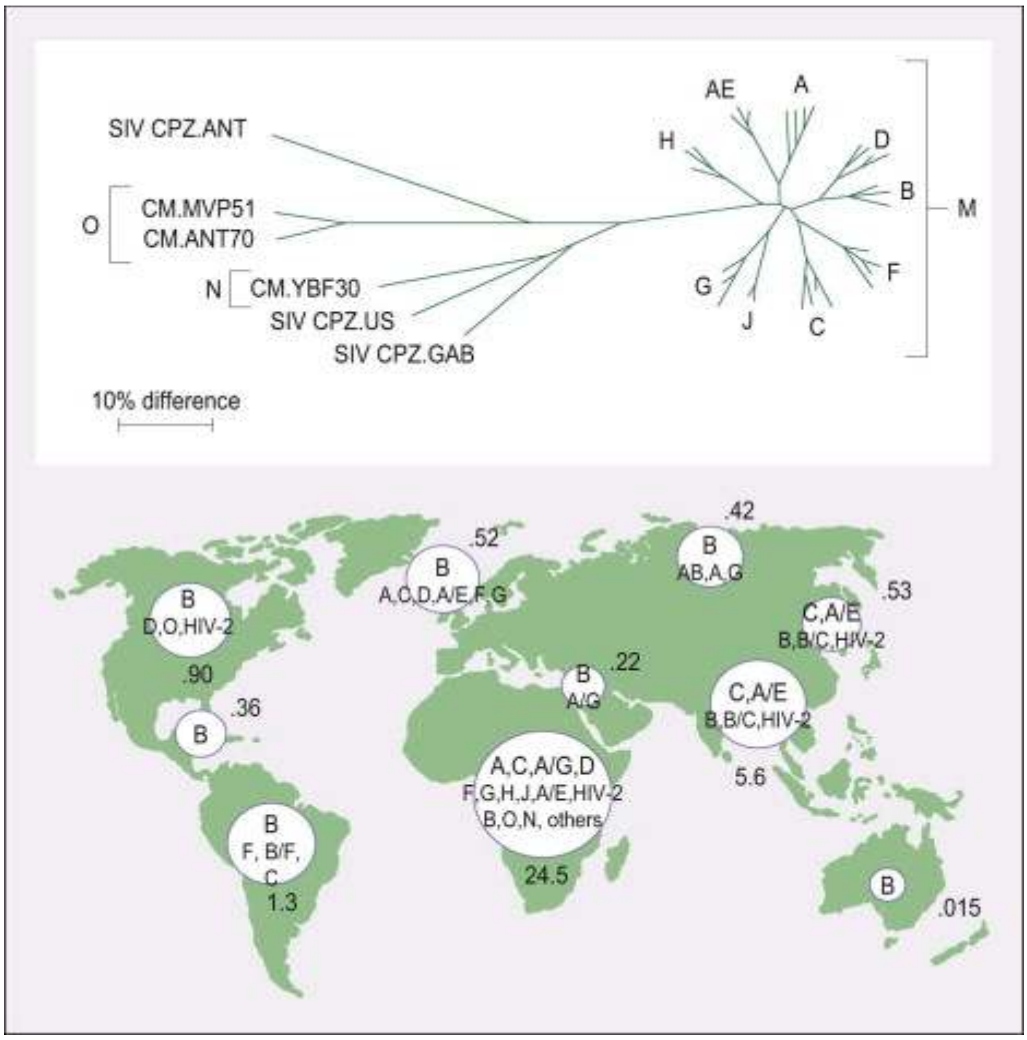


Fig -1

Epidemiology.

HIV infection is global pandemic. According to UNAIDS there will be 33.3 million Individuals where living with HIV infection at the end of 2009. About more than 95% of the people living with HIV/AIDS resides in low and middle income countries, of that 50% will be female and 2.5million are children less than 15 years. In 2009 the global AIDS deaths was totalled around 1.8million that includes 2, 60,000 children less than 15years. HIV epidemics have occurred in waves in different regions of the world. More than 2/3rd of all peoples with HIV infections live in sub Saharan Africa that has only 10-11% of the world's population, within that the southern Africa is worst affected. Heterosexual exposure is the most common mode of transmission there.

In east, south and south east Asia are mostly affected. Among the Asian countries, Thailand has an adult seroprevalance rate of >1%. In Bangladesh and Pakistan the prevalence has increased very much. In United States as of Jan 2010, the total cases estimated were 1,108,611. Around 1.1 million individuals were living with HIV infection of which 21% are unaware of the infection. Around 48% were men who have sex with men [9].

An estimated 56,000 individuals are newly infected each year. The HIV Estimates according to the UNAIDS REPORT 2012, in India alone was 20.89 lakh in 2011. The sexually active age-group (15-49) prevalence showed a declining trend from an estimated level of 0.41% in 2001 to 0.27% in 2011. Even Though The Estimates Are Declining India continues to be the third largest population of (PLHA) people living with HIV/AIDS, only behind South Africa and Nigeria[9].

The first AIDS case was reported from Chennai in 1986 and has been reported since then across all the states and union territories. In 1992 the Indian government launched the National AIDS Control Programme (NACP-I) in an all out national effort to control the spread of the epidemic. The programme was a comprehensive one with more emphasis on Prevention and care.

This process led to the formation of the National AIDS control board and subsequently National AIDS CONTROL Programme through which the Indian government started the free distribution of free HAART medications since 2004 and which has continued with amazing success till date .

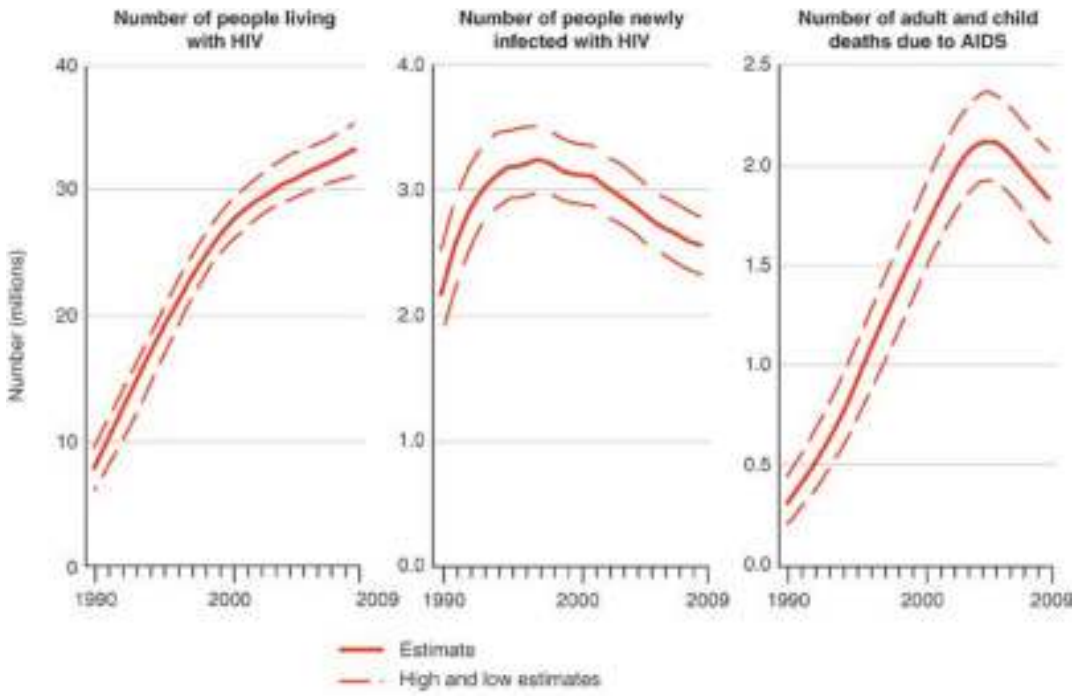
Indian government started the free programme on 1st April 2004 in the high prevalent states of Karnataka, Tamilnadu, Andhrapradesh, Maharashtra, Manipur and Nagaland. In March 2013, there were around 18 lakhs People Living with HIV (PLHIV) documented in the registry of the 400 ART Centres across India .

Currently about 6.5 lakhs are on first line ART and along with this there are about 840 Link ART Centres (LAC) functioning with the primary objective of dispensing the ART drugs and monitoring their side effects and also for treatment of minor opportunistic infections .out of these about 154 LAC have been upgraded to as LAC plus centres to provide additional Pre ART services.

The estimated incidence of new HIV infections annually in India is around 1.16 lakhs among adults. The total number of PLHIV is estimated grossly at 21 lakhs in 2011. the break up is that the children (<15 yrs) account for 7% (about 1.45 lakhs). About 39% (8.16 lakhs) of women are infected and these estimated numbers of PLHIV have shown a steady declining trend from 23.2 lakhs in 2006 to 21 lakhs in 2011[10].

About 53 % of cases were seen amongst the four high prevalent states of South India excluding Kerala. The high risk groups and their vulnerability profiles have been different in different parts of the country

and they have been driving the epidemic throughout the country. Various prevention and treatment strategies and have manage to contain the epidemic as evidenced by the reduction in the overall incidence of new infections besides declining trend in the AIDS related morbidity and mortality [10].



Morphology of HIV

The virus has an icosahedral structure which contains numerous external spikes which is formed by the major envelope proteins called the external GP 120 and the second protein called the trans membrane protein GP 41. The virus gives out virion from the surface of the infected cells and it also incorporates various proteins into its lipid bi-layer namely the

Major Histocompatabiliity Proteins (MHC) Class I AND II antigens[11].

The structure of the virus is

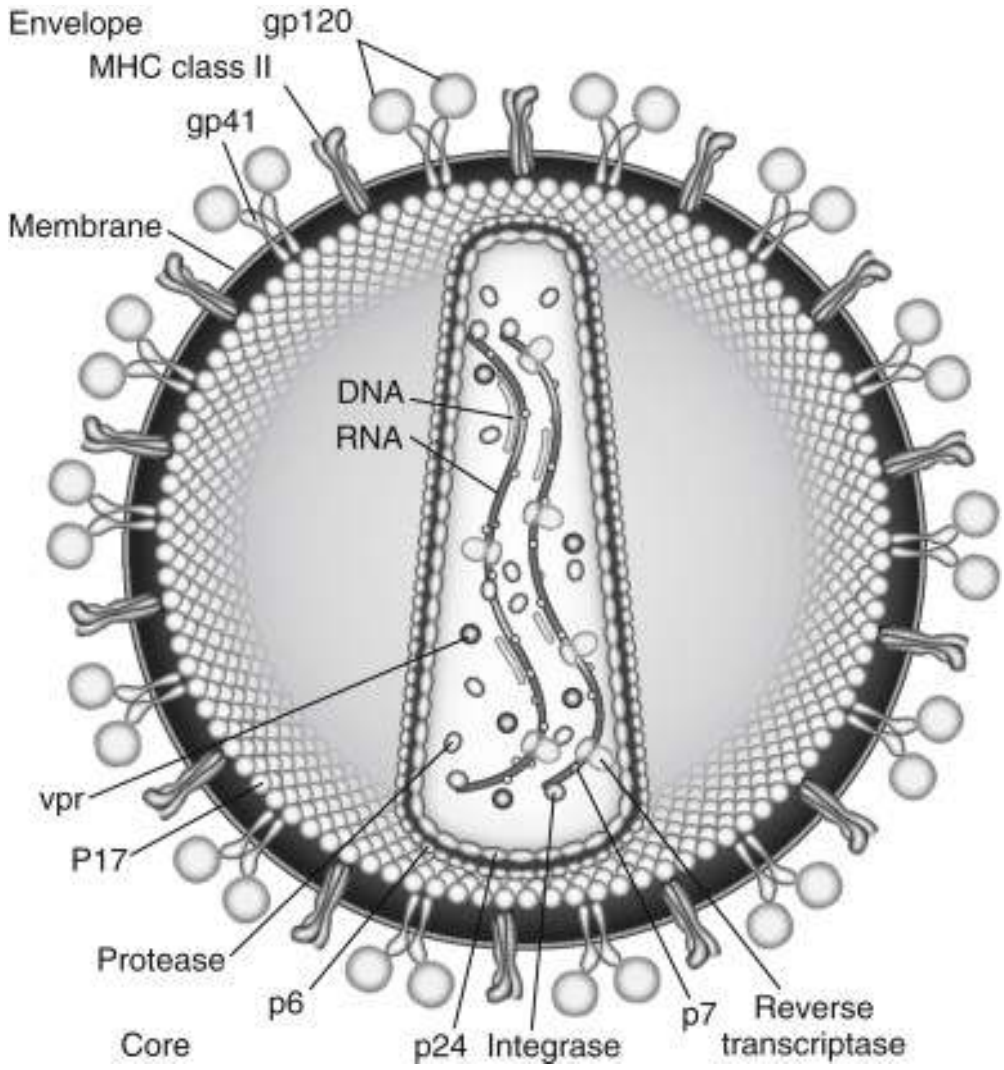


Fig -2

Replication of HIV

Replication phase I

This starts with the attachment of the virus to the CD 4 (cluster differentiated) receptor and to the Chemokine receptor (CCR4/5) that is present on the surface of the cell. There are two major co-receptors for HIV-1 which belong to the family of the seven trans membrane domain G protein coupled cellular receptors and are called the CCR5 and CXCR4. There is also another receptor on the dendritic cell called the (DC-SIGN), that binds with a very high affinity to the HIV gp120 envelope protein, and this process facilitates the dendritic cell to bind to the CD4 cells when they engage with the CD4 cells. There occurs endocytosis which causes the entry of the virus into the cell followed by the fusion of the envelope of the virus with the plasma membrane of the cell that has been targeted .The next process in the replication is the uncoating in the cytoplasm of the nucleocapsid which happens when there occurs a protein called the virion associated cyclophilin A whose presence actually facilitates the process[12] .The next process is the reverse transcription of the viral Ribose nucleic acid (RNA) to the double stranded viral Deoxy ribose nucleic acid (DNA) After this process the viral DNA enters the nucleus of the cell as a pre- integration complex that contains the genes vpr and integrase .There are certain cellular factors that can block the

progression of infection at this point of the replication cycle .The cytoplasmic Protein called the TRIM 5 which has been found in the rhesus macaque monkeys has been found to block the simian immunodeficiency virus (SIV) replication soon after the virus fuses with the host cell. Another family of proteins called the APOBEC family of cellular proteins have also been to inhibit the progression of virus soon after the virus has entered the cell. These proteins have been found to bind to a nascent reverse transcripts and in that process deaminate the viral cytidine thereby causing hyper mutation of the HIV genomes. But the confusion remains which among the two processes namely binding to the nascent reverse transcripts or the hyper mutations that kills the virus. It Has been documented that the HIV can escape from the APOBEC proteins with the help of the vif gene which cause a proteasomal degradation of the targeted proteins thereby protecting the virus from the host proteins . After the process of integration the integrated linear DNA (now termed the provirus) serves as the template for viral transcription.

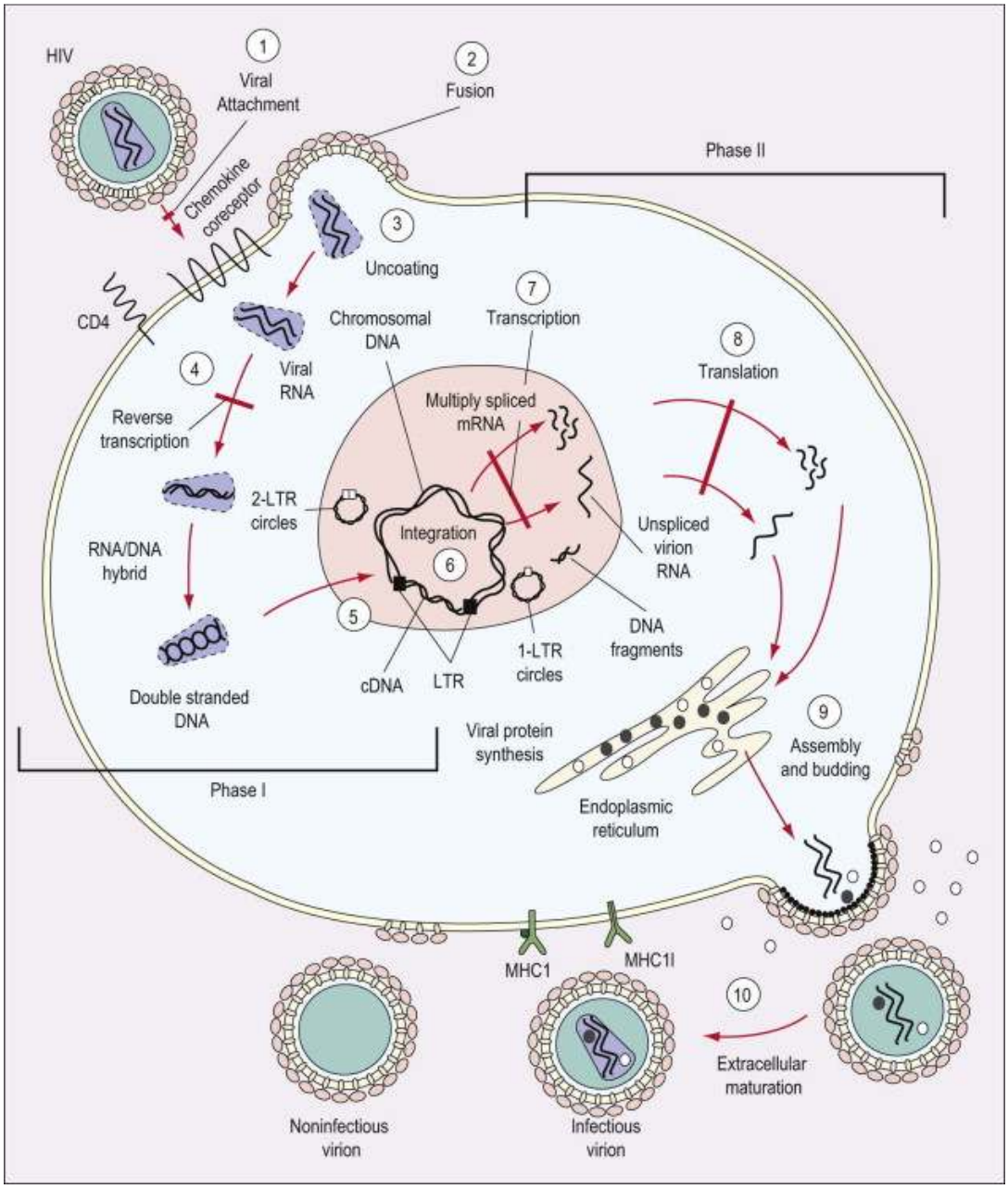

HIV
1
Viral Attachment
2
Fusion
Phase II
Chemokine coreceptor
3
Uncoating
CD4
7
Transcription
Chromosomal DNA
8
Translation
4
Viral RNA
Reverse transcription
Multiply spliced mRNA
2-LTR circles
Integration
6
Unspliced virion RNA
RNA/DNA hybrid
5
DNA fragments
1-LTR circles
cDNA
LTR
Double stranded DNA
Viral protein synthesis
9
Assembly and budding
Phase I
Endoplasmic reticulum
MHC1
MHC1I
10
Extracellular maturation
Noninfectious virion
Infectious virion

Figure - 3

Replication phase II

The process of transcription of the proviral DNA template yields a genomic viral RNA and a further process called the splicing of the messenger RNA takes place which creates spliced viral mRNA species which encode for the accessory viral proteins and there are also unspliced viral mRNA that are present which encodes for the viral structural poly proteins[13].

All the transcripts are subsequently exported to the cytoplasm for a process called the translation. The viral mRNA is subsequently translated into the proteins which further undergo post translational modification processes like phosphorylation, glycosylation, cleavage, and myristoylation and then occurs the assembly of all the proteins in the endoplasmic reticulum.

The viral polypeptides are formed by the gathering of the HIV proteases, vRNA and other constituents of viral core at the membrane described as the *lipid rafts* of the cells that have already accumulated the proteins (gp120 and gp41)[14]. Budding occurs in the lipid bilayer of the host cell membrane. where the immature virion core acquires its external envelope and it further undergoes proteolytic maturation in the extracellular milieu.

The mature virion is formed by the subsequent cleavage of the gag- pol precursor by virally encoded protease . The entire viral life cycle is controlled by numerous regulatory proteins and each of these are a potential target for therapeutic intervention. Until now the targets have been the enzymes the reverse transcriptase, protease, and the integrase and also the virus –target cell binding and fusion have also been successfully targeted to get maximal benefit in disrupting the viral replication and in keeping the infection at bay[15].

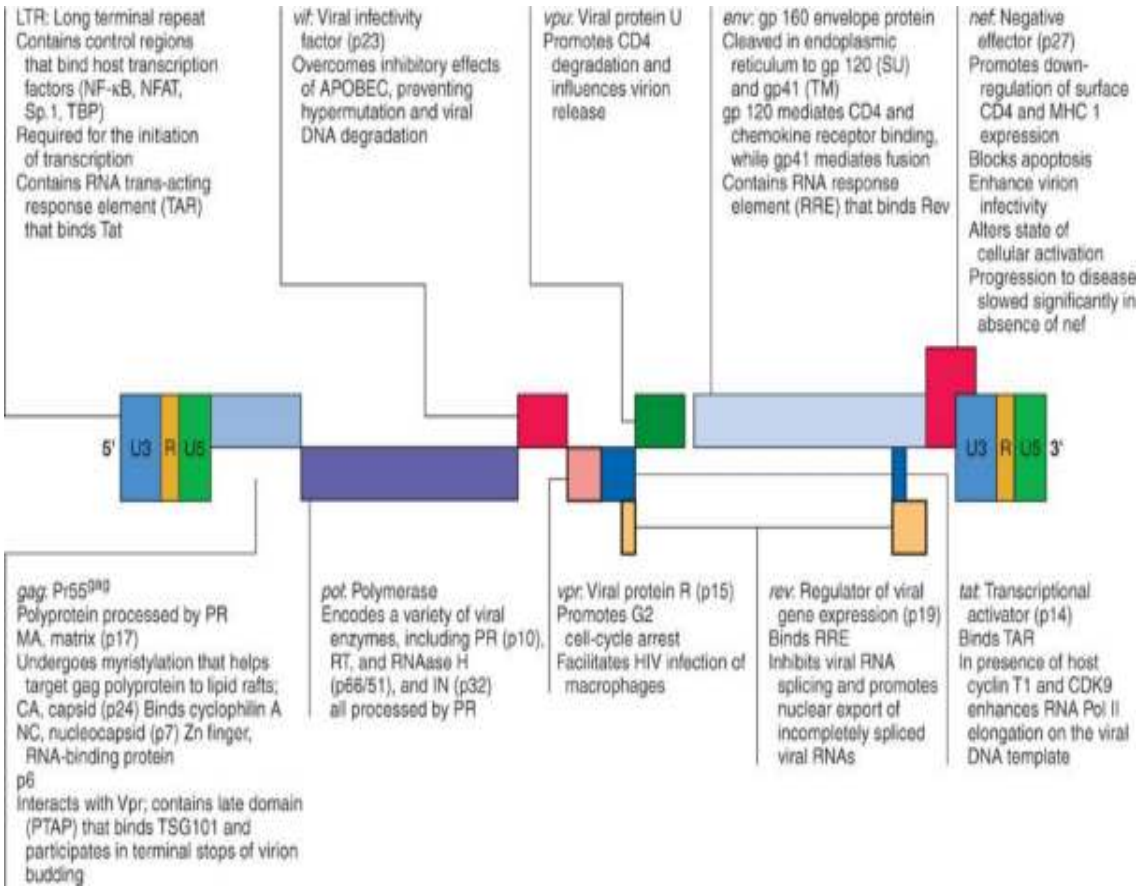
The HIV Genome .

There are various genes in the HIV-1 (which is structurally more complex when compared to HIV 2) that encode for various proteins and enzymes. They are the *gag* which encodes for the core proteins and which also includes the p24 antigen. The pol gene encodes for the protease which processes the viral proteins, reverse transcription, and integration[16,17].

Then the *env gene* codes for the structural envelope glycoprotein. There are 6 other genes in HIV 1 namely *vif*, *vpr*, and *vpu, tat*, *rev*, *nef*, which codes for regulation of viral gene expression [18,19] . Besides these genes are the regulatory elements of gene expression called the long terminal repeats (LTRs).

The major difference that exists between HIV-1 and HIV-2 lies in the genome in the fact that HIV-2 does not have the *vpu* gene but has an extra *vpx* gene which is not seen in HIV-1. The figure - 4 shows the HIV proviral genome [20-24].

Figure - 4

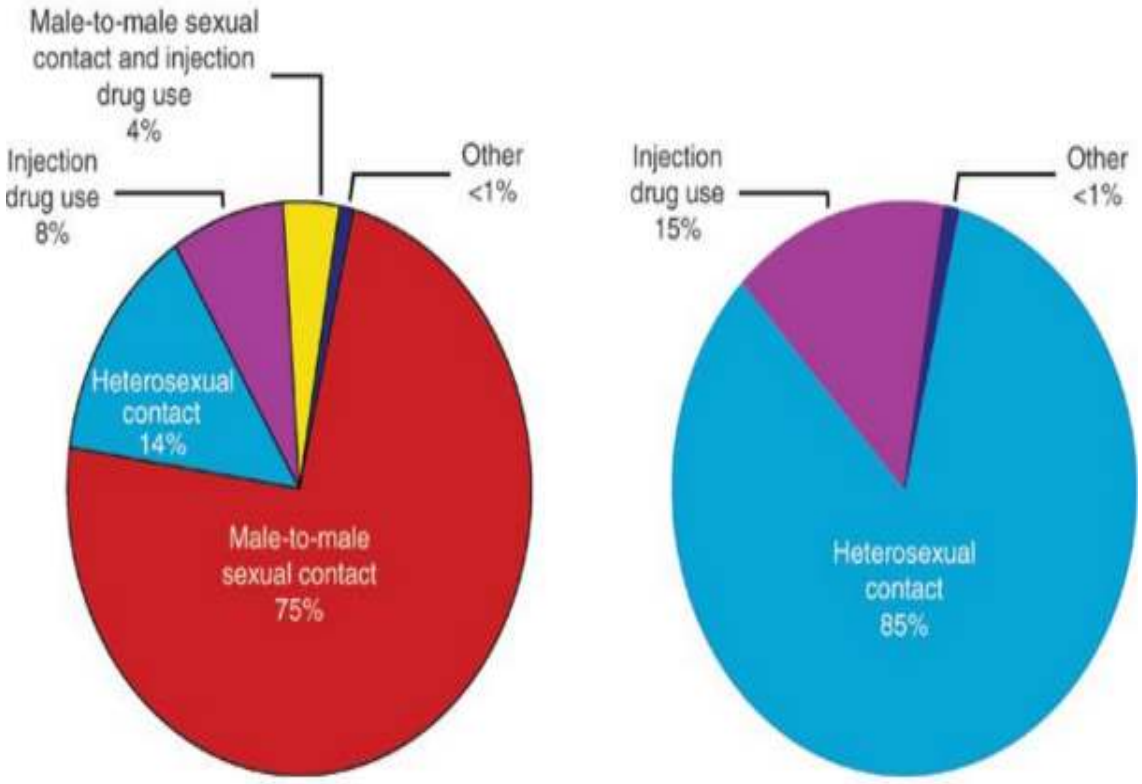


Transmission Dynamics.

HIV is transmitted by various means namely by Blood (either by blood transfusion, or by the reusage of IV needles/syringes), by the transfer of Deep body fluids (genital secretions, tissue, breast milk) in between two persons that can be either heterosexual or homosexual and lastly by the vertical transmission from mother to child that can happen at any time during and after pregnancy. It has also been documented that there is no risk of transmission through mosquito bites. The Risk of HIV infection following a single exposure to an HIV infected source are as follows, if it is following a Puncture of a health care worker by a contaminated needle the risk is 1:313, whereas if is it due to a usage of contaminated injection equipment it is about 1:149 and the greatest risk is following a blood transfusion where the risk is 1:1.1 (>95%), the greatest following a single exposure to a HIV infected source .

The Risk of HIV infection following a single sexual exposure to an HIV infected source are as follows .Following **a** Receptive anal intercourse the risk is 1:125 to 1:31, wheras following a Receptive vaginal it is about 1:2000 to 1:667. It is about 1:3333 to 1:1111 following an Insertive vaginal or anal intercourse, and there are no published estimates for oral sex[25]. The figure - 5 depicts the prevalence of the various transmission risks

Figure - 5



HIV is and has been predominantly a sexual transmitted disease .The various frequencies of transmission per coital act have been discussed above .It has been demonstrated the seminal secretions contain HIV virus both within the cell free material and amongst the infected cellular material.

During a coital act a direct inoculation of the virus into the blood might happen if there exists patients with both the vagina and anal canal tears and the infection of the target cells namely the Langerhans cells

might happen in the mucosal layer in cases where there is absence of trauma.

It has been found that the Insertive anal intercourse is also equally infective when compared to the Insertive vaginal intercourse. It has also been found that the virus can be transmitted either way to both the partners during a vaginal intercourse.

Various studies have shown that the male-to-female HIV transmission has more risk than the reverse. These might be due to the increase time duration of exposure of the vaginal, cervical mucosa and the endometrium to infected seminal fluid. When compared with this during a coital act among heterosexuals there is only a brief exposure of the genital organs to the infected vaginal fluid.

Other factors that have been studied in depth are the presence of co-existing Sexual Transmitted diseases ,and whether the male partner has been circumcised and the presence and absence of coexisting genital ulcers.

The infections like Treponema pallidum, Hemophilus ducreyi, herpes simplex, Chlamydia trachomatis, Neisseria gonorrhoea and trichomonas vaginalis have been associated with high transmission rates of HIV infection.

It has also been proved beyond doubt that the treatments of these sexually transmitted diseases have reduced the transmission of HIV[26]. The figure 6 summarises the risk of transmission in non occupational exposures .

Figure 6

Type of exposure (from a source known to be HIV positive)	Risk of HIV transmission per exposure
Accidental needlestick injury	0.2%-0.4%
Mucosal membrane exposure	0.1%
Receptive oral sex	From 0 to 0.04%
Insertive vaginal sex	≤ 0.1%
Insertive anal sex	≤ 0.1%
Receptive vaginal sex	0.01%-0.15 %
Receptive anal sex	≤ 3%
IDUs sharing needle	0.7%
Transfusion	90-100%

The quantity of HIV viral load and the CD4 cell counts have significant role in the transmission dynamics. The higher the viral load higher the transmission and higher the CD4 cell counts, lower the transmission rates.

Pathogenesis.

Following exposure to and infection with HIV-1 there is a variable period of viral replication in mucosal and lymphoid tissue that drains the inoculation site and has been codified as Fiebig Stages I–VI [27].

The appearance of infectious viremia, as determined by infectious blood donation studies (time 0), marks a consistent sequence of virological and serological events.

There is a rapid rise in detectable plasma viral RNA at 3–8 days (Stage I), followed by p24 antigen and viral DNA by 7–14 days (Stage II for p24 antigen alone) with viral RNA and p24 antigen peaking between 20–30 days.

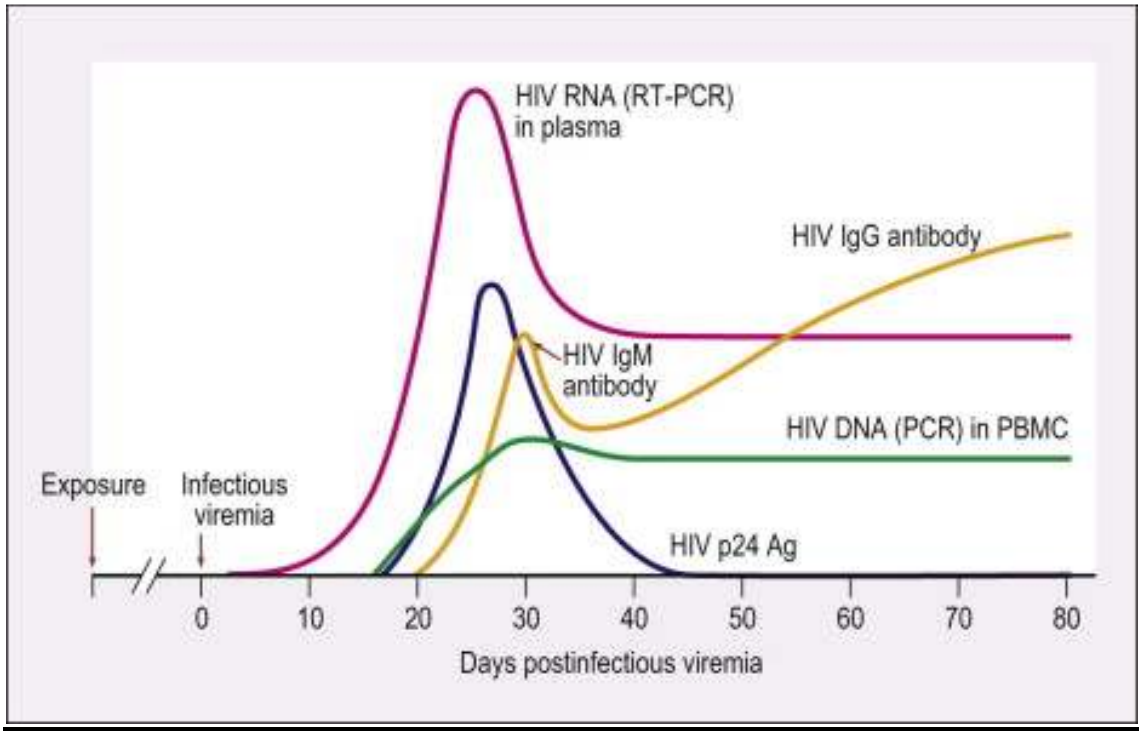
The detection of infected peripheral blood mononuclear cells (PBMC) coincides with the detection of PBMC-associated viral DNA.

Anti-HIV IgM appears between 10–17 days post-viremia (Stage III), peaks and is followed by a slow rise in the level of anti-HIV IgG [28,29].

An indeterminate Western blot develops by 15–23 days (Stage IV), develops a confirmatory pattern with p24 core and env antibody but no antibody to the p31/32 integrase by 47–130 days (Stage V) and then is followed by anti-p31/32 thereafter (Stage VI).

During the first 4 months of infection, the level of anti-HIV may not be detected by less sensitive immune-enzyme (EI) assay formats (so-called 'detuned' assay); thus, the detuned EI assays may be used to determine point incident infection rates.

Over the ensuing 4–6 months, seroconversion is associated with the suppression of the viral replication to a stable viral RNA level or 'set-point' that is prognostic of the risk for disease progression in the untreated patient[30,31].



PERSISTENT VIRAL REPLICATION:

Following the primary infection the humoral and cellular immune responses are activated but the virus finds a way to escape the immune mediated clearance and stays in the body itself and is never completely eliminated from the body. Within a median of ten years a chronic HIV infection sets in which is a hallmark of the HIV disease after which the patient becomes ill. There occurs a persistent viral replication continuously in the body and this can be detected by highly sensitive assays in the circulation and the lymphoid tissues. This persistent replication exponentially increases the viral load alongside the destruction of the immune system [32]. This is different from the Chronicity seen in HCV and HBV infection, where the immune system is not the target for these viruses and remains intact without manifestations of the immunodeficiency syndrome complex.

Mechanism of Immune Evasion

There are various mechanisms by which the virus evades the immune system .The main one is by establishment of continuous viral replication with a varied capacity to mutate and recombine. The CD8 cytotoxic T cells that are produced and expanded during primary HIV infection are deleted and made dysfunctional due to the persistent viral replication[33]. The second mechanisms by which the virus escapes from

immune system is by the lack of recognition of the surface of the HIV cell by NEF proteins as a result of the down regulation of the HLA Class I molecules.

The third mechanism that has been demonstrated is the evasion of the HIV from the Neutalisin responses by causing hypervariablity in the primary sequence and by glycosylation and masking of the neutralising epitopes[34] .

One another important mechanisms by which the virus evades is the collection of infected cells in certain places called reservoir sites which are also called immunologically privileged sites and the Central nervous system is one such site . Since HIV primarily infects CD4 cells and so this loss of those cells damages the immunologic control. This evasion of HIV from the immune response cannot be eliminated by the HIV-1 specific cytotoxic cells, so HIV succeeds in putting strong basement creating a state of chronic infection[35].

HIV reservoir sites: Obstacles to the Eradication of Virus

As mentioned above there is a constant pool of latently infected resting CD4 cells which causes a persistent reservoir of cells containing the virus. There exists two latencies that are called the pre and post integration latencies. This pool of latently infected cells persists even if

the viral load is less than 50 copies as a result of the effect of the potent anti - retroviral treatment, and they can persist to produce replication competent virus at a later date. The reservoir sites for the HIV infected cells are the lymphoid tissue, the peripheral blood and CNS. These reservoir sites serves as the major drawbacks in the complete eradication of the virus from the human body[36,37].

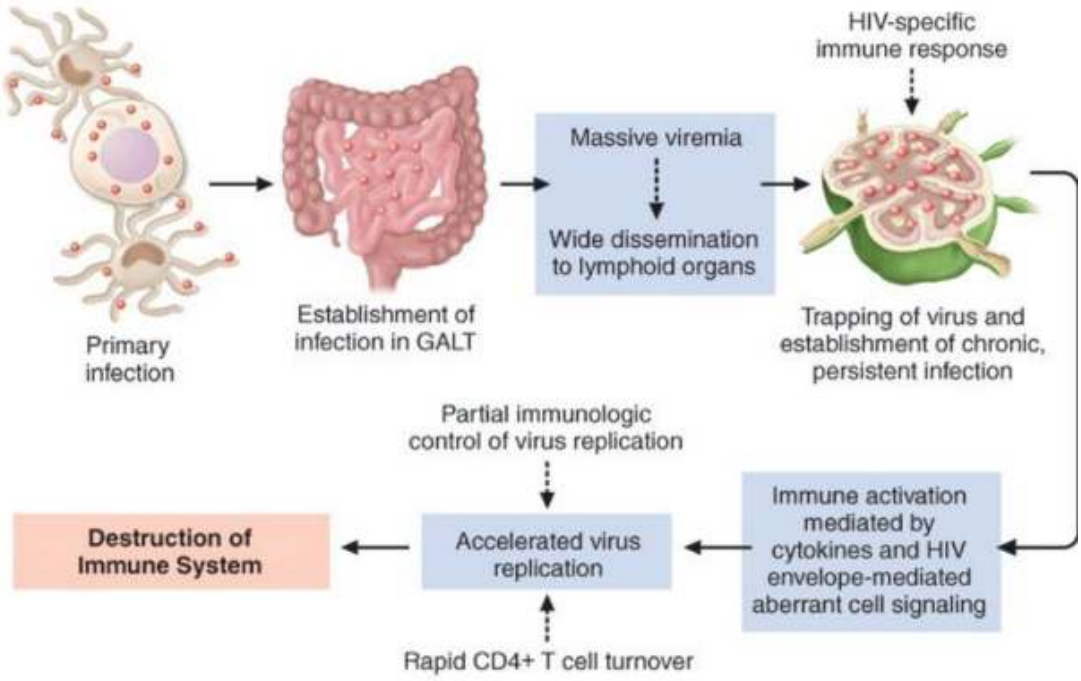
Early events in HIV

The multiplication, establishment and progression of HIV infection happens at the lymphoid tissues. The viral replication mainly occurs in lymphoid tissues. The cellular response and immune activation that follows the viral replication in the lymphoid organs causes the lymphadenopathy. It has been shown that the Gut Associated Lymphoid Tissue (GALT) is the site of earliest viral replication [38].

In the early stages of HIV, the architecture of the germinal centre is mostly preserved. The follicular dendritic cells trap the antigens and present it to the B cells which is the normal physiologic function. In case of HIV the trapped virions causes secretion of pro inflammatory cytokines such as IL1 beta, TNF alpha, IL6 which will up regulate the viral replication.

CD4 helper cells which goes into germinal centres are susceptible to infection by these trapped virions. So in HIV infection the normal physiologic function of immune system is affected. As the disease progress the germinal centre which was preserved early will undergo disruption and swelling, finally to cell death. In the advanced stage of disease there is complete disruption and resolution of the follicular dendritic cell and finally goes into a stage of burn out.

This destruction of lymphoid tissue affects both the inability to control replication and inability to make immune response [39].



ROLE OF CYTOKINES

Cytokines which are the components of immune system play an major role in regulating the HIV-1 expression. The cytokine involved in the induction and enhancement of HIV expression are IL 1, IL 2, IL 3, IL 6, IL18, IL12, TNF alpha, TNF beta, GM-CSF. TNF alpha, IL 1 beta and IL 6 are the potent inducers of HIV expression. IFN alpha, IFN beta, IL 32 suppress the HIV replication[40].

While TGF beta, IL4, IL10, IFN gamma will induce or suppress HIV expression depending on the system involved. The elevation of TNF alpha and IL6 are demonstrated in plasma and CSF while TNF alpha, IL 1 beta, IFN gamma ,IL 6 are demonstrated in the lymph node[41]. HIV replication is controlled by endogenous cytokines which acts synergistically in an autocrine and paracrine manner. Finally the secretion of some pro inflammatory cytokines is a result of aberrant immune activation seen associated with HIV infection[42,43].

Lymphocyte turnover in HIV infection.

There is an increased turnover in the lymphocyte counts when there is an HIV infection inside the body. The turnover decreases with the initiation of the cart. The high cell turnover correlates with increased cell death as well.

The thymus also plays an important role which has been demonstrated by the increase in the levels of T cell receptor excision circles (TRECs) after the initiation of HAART. The levels of TREC correlates with the changes in thymic output and changes in T cell turnover. An increase in thymic output or a decremental response in T Cell turnover causes an increase in the levels of TREC .

Role of Receptors and Co-Receptors.

The HIV-1 uses the two major co-receptors CCR5 and CXCR4, along with CD4 receptor to bind ,fuse and to the enter the target cells; The co receptors share their receptor space with some endogenous Chemokines .*R5 viruses* are Strains of HIV which utilizes the CCR5 co-receptor and X4 Viruses are those which utilize the CXCR4 co receptor. There are also certain strains that utilize both the CCR5 and CXCR4 and these are called the dual tropic viruses and they are also called as *R5X4 viruses*.

There are some natural ligands like the CC-Rantes (CCL5,CCL4,CCL3) which are the natural ligands for the CCR5 receptor and these play a major role (inhibition of binding) in blocking the entry of R5 viruses into the body the SDF-1 plays a similar role with CXCR4 thereby blocking the X4 viruses. During the early stages of HIV disease the transmitting virus is invariably an R5 virus and In 40% of HIV-

infected individuals, transition to a predominantly X4 virus is linked to a rapid progression of disease. At least 60% of affected patients progress in their disease status while predominance of an R5 virus is being maintained[44].

Mucosal Transmission of HIV.

A recently detected receptor is the integrin alpha4/beta7 which plays an important role not in the binding to CD4 cells but to the mucosal surfaces in the gut and the genital tract [45]. It has been demonstrated that the virus which binds to this receptor during a sexual exposure binds more efficiently than a virus which diversifies over time by mutation.

Cellular Targets for HIV.

The various cell lines that can be infected with the HIV are the CD4+ T lymphocytes,CD4 cells of monocyte lineage, Circulating dendritic cells, Epidermal Langerhans cells, and the thymic precursor cells, though they were assumed to be negative for CD3, CD4, and CD8 ,they actually express low levels of CD4 and hence can be infected with HIV.

CD4 Dysfunction and Depletion.

There are certain direct and indirect ways in the dysfunction thereby causing the depletion of the CD4 cells .The direct mechanisms

are the viral budding due to a loss in the plasma membrane integrity, unintegrated viral DNA accumulation and interference with processing of the cellular RNA, autofusion events involving the intracellular glycoprotein 120 and CD4, and lastly the formation of the Syncytia. The various indirect mechanisms also exist and they are Autoimmunity, Activation-induced cell death and the damaging of antigen coated cells [46,47].

Mechanisms for CD4+ T-cell killing	
Direct	
Direct cell killing	•Disruption of cell membrane by massive viral budding •Syncytium formation •Accumulation of unintegrated viral DNA •Interference with cellular RNA processing •Elimination of HIV-infected cells by virus-specific immune responses
Indirect	
Apoptosis	•Alterations of signaling mechanisms within cells may prime cells for apoptosis •Fas-dependent and Fas-independent pathways may be triggered by HIV •Increased susceptibility of uninfected bystander cells to apoptosis
Immune attack of uninfected cells	•Innocent bystander phenomenon whereby free viral antigens on the surface of infected cells (gp 120 env) may bind to the CD4 receptor of uninfected cells making them targets for both antibody and cell mediated destruction •Molecular mimicry between HIV-1 envelope constitutents and host proteins may result in autoimmune responses
HIV-inhibited hematopoiesis	•Infection of CD34+ stem cells •Viral proteins and HIV-induced cytokines impair CD34+ survival and clonogenic potential
Thymus damage	•Inhibition of thymopoiesis by infection of immature CD4 and CD8 cells (CD3-CD4-CD8- "triple negative" cells and CD3-CD4+-CD8-progenitor cells) •Damage to the thymic epithelial cells and disruption of thymic microenvironment contributes to the failure of CD4+ T-cell regeneration
Lymph node damage	•Destruction of geminal centers and lymph node structure
T-cell anergy	•Block of T-cell proliferation after contact with soluble HIV proteins leads to reduced clonal expansion

GENETIC FACORS IN HIV PATHOGENESIS

Several genetic variations have been now identified in human beings that influence the risk of acquiring HIV, rate of disease, progression, virological control and immune response. There are polymorphisms identified in genes in the MHC locus, chemokines, cytokines and other host factors. Recently they have identified polymorphisms within the HLA-B and HLA-C which is associated approximately 15% variation in viral load during the asymptomatic period[48].

In some individuals MHC Class I and class II will predispose them to an immunopathogenic response particularly in some tissues like CNS, Lungs or against some HIV infected cell types such as macrophages, dendritic cells, Langerhans cells. There is increased risk of transmission of HIV infection among heterosexual Zambian couples who have an alleles sharing at HLA-B locus. Also founded HLA heterozygosity for class-I loci are associated with delayed onset AIDS in HIV, where as in HLA homozygosity it was a reverse.

Another gene called TAP gene plays an important role in the outcome of HIV -1infections. It is also noted that individuals with haplotype 8.1 also been correlated with rapid decline in CD4 T cells.

Recent studies shows the single nucleotide polymorphism (SNP) in the killer immunoglobulin like receptor (KIR) was found to be strongly associated with rapid progression to AIDS. The best example for a genetic factor which influences HIV infection and pathogenesis is related to the gene which codes for CCR5 the major HIV co-receptor.

There are individuals who remains uninfected even though after repeated sexual exposure to HIV even though in high risk situation. These individuals were found to have high resistance with R5 strains of HIV-I but they were readily infected with X4 strains. On various analysis these individuals inherited a homozygous defect in the gene that encodes for CCR5. They have a homozygous defect for CCR5 32 allele 20% of European individuals are heterozygous for CCR5 32 allele and has partial resistance or a delayed disease course. Cohort studies from western, central Africa and far east Asia has absent CCR5 32 allele.[21]

IMMUNE RESPONSE TO HIV

Both humoral and cellular immune are very essential in HIV -1. These responses are directed against variable antigenic determinants of HIV.

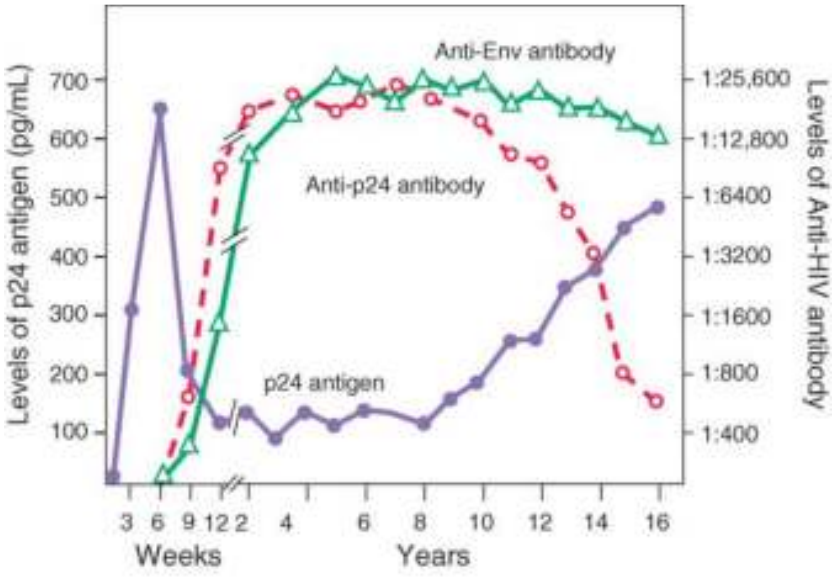
HUMORAL IMMUNE RESPONSE

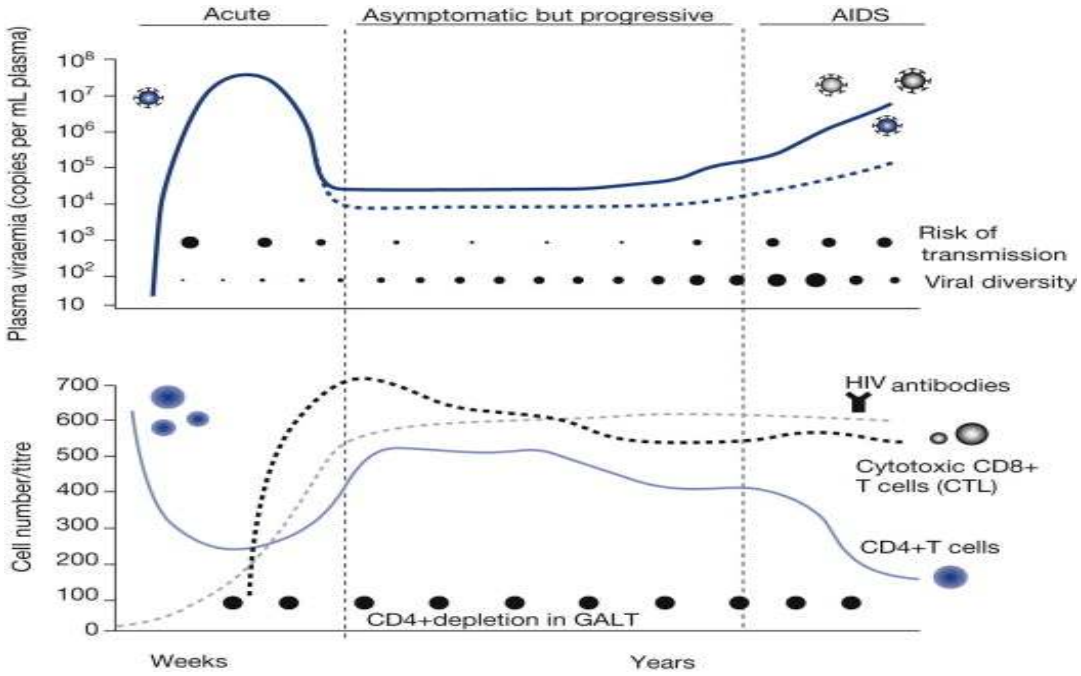
Usually the antibodies to HIV will appear within 3-6 weeks .Almost invariably within 12 weeks of primary infection. Usually the neutralizing antibodies appear after the initial decrease in plasma viremia. The antibodies that are detected first are those against envelope gp41 followed by gag protein p24 and gag precursor p55. gp 120 and gp 41 are the only enveloped proteins that elicit neutralizing antibodies.

The neutralizing antibodies appears in first 6 months, but viruses escapes these antibodies. There are 2 types of neutralizing antibodies one is type specific antibodies that appears early in the infection and they neutralize the viruses of a given strain.

The other one is group specific neutralising antibodies that appears late in infection, which they neutralize a wide variety of HIV. There are the 2 types of group specific neutralising antibodies one directed towards the CD4 binding site of gp120 and those binding to proximal region of gp41. Antibodies directed against gp120 and gp41 also participate in antibody dependent cellular cytotoxicity mediate killing of HIV infected cells.

There is a entity called bystander killing in which the anti gp130 antibodies kills uninfected CD4 T cells complement also play an important role in humoral immune response.





CELLULAR IMMUNE RESPONSE:

Cellular immunity is mediated by CD4 and CD8 and the CD4 cells helps the HIV specific B cells and CD8 T cells in the direct killing HIV infected cells. CD8 T lymphocytes cause the lytic destruction of target cells.

There are 2 types of cytotoxic T lymphocytes the first type directly lyses the target cells without prior in vitro stimulation, the other type reflects the precursor frequency of cytotoxic T lymphocytes. It is also found that there is direct relation between the levels of CD8 T cells Capable of producing IFN-gamma to HIV antigen and RNA levels. Some of the forms of cell mediated immunity to HIV described are CD8 T cell mediated suppression of HIV replication, antibody dependant cellular cytotoxicity and NK cell activity [47].

FACTORS INFLUENCING HIV DISEASE PROGRESSION

HOST FACTORS

Old age peoples have a rapid progression of the disease, but it was an independent predictor in IV drug abusers. The gender do not have any major influence over the disease progression. Ethnicity also doesn't have any influence.

It has been studied that 5% of HIV patients will be clinically stable for about 10 or more years after seroconversion and they are called as long term survivors or long term non progressors.

COFACTORS

HIV patients with CMV seropositivity were found to have 2-4 fold higher risk of disease progression. This states that CMV is a cofactor in HIV patients which leads to disease progression. There is no clear cut data that EBV ,HHV6, hepatitis B, acts as a cofactor.

OTHER POTENTIAL COFACTORS

To infect a cell HIV needs CD4 receptor. Another receptor needed to bind and infect the cells are called Chemokine receptor named CCR5. In many individuals this CCR5 carry a mutant gene delta32 deletion. If the persons are homozygous to delta32 then they found to have less risk for the infection with HIV. There are also certain factors cytokines called the Stromal derived factor-1 (SDF-1) which has affinity to the CXCR4 receptor. Individuals having mutation of the gene that produces SDF-1 are more resistant to infection. Association between smoking and CD4 lymphocyte loss or more rapid disease progression were founded in some studies. In developing countries it was studied that malnutrition may accelerate the HIV disease progression [49].

MARKERS FOR HIV DISEASE PROGRESSION

Certain measurable traits help in disease staging and predicting susceptibility to opportunistic infections. Beta2 micro globulin is a non specific marker of immune activation. High titre levels are seen in association with disease progression. Also higher levels are found in various viral infections and in patients with lymphoma.

Neopterin which is derived from macrophages and B lymphocytes is estimated using liquid chromatography and radio immunoassay predicts the disease progression. Other conditions having elevated neopterin levels are collagen vascular disorders, malignancies and some infection.

Other traits that have some significant role are the soluble CD8,s il-2r ,anti p 24 antibody ,anti gp 120 antibody and p24 antigenemia. High levels of s IL -2R are found in patients with AIDS and has a negative correlation with CD4 cell counts . Soluble CD8 is an early marker of infection and levels correlate with number of CD8 lymphocytes. Poor prognosis is seen in patients with declined anti p24 antibody[49] .

Absence of the antibody is related to disease progression. p 24 antigen is transiently seen in acute stages of HIV infection and in latent stages of HIV disease. A low CD4 count with p24 antigenemia is a very

strong predictor of disease progression. Some of the HIV isolates who have syncitium inducing capacity are seen in later course of the disease. The increased percentage of CD38 positive CD8 T cells reflects a high viral load which indicates the disease severity[46].

DIAGNOSIS OF HIV INFECTION

Screening tests for the detection of HIV-1 is by ELISA which has a sensitivity of more than 99.5%. But however the confirmatory test in the western blot which detects multiple antibodies to HIV proteins. Other tests are DNA PCR, RNA PCR, b DNA assay and P24 antigen capture assay.

The p24 antigen capture assay measures HIV-1 core protein in an Enzyme linked immuno assay based format. The detection of HIV RNA is by the PCR amplification of the complementary DNA generated from viral RNA which is also called the target amplification.

Another sensitive measurement of HIV RNA is by measuring the branched DNA (bDNA). Measurements of HIV-1 RNA by isothermic nucleic acid amplification with internal controls called the nucleic acid sequence–based amplification (NASBA) is also a very sensitive method.

GUIDELINES BY NACO FOR HIV DETECTION [10]

STRATEGY I

This is a strategy which is used for blood donor screening. A single ELISA test is done, and if it is found to be negative the donor serum is considered free of HIV, if it is positive it is not informed to donors.

STRATEGY II

It is mainly used for the surveillance and diagnostic purposes. Two tests are done and the first test usually an ELISA is negative then the sample is considered negative, in contrast if the first test done shows a positive result then the second test is done and is reported as positive when both the two tests are positive.

STRATEGY III

It is used in diagnosing HIV in asymptomatic individuals. This strategy uses a third reactive test which is needed before reporting a positive result. For those symptomatic persons the sample must be show a positive reaction with 2 different kits and for an person who is asymptomatic and the same should be positive with3 different kits.

CLINICAL STAGING OF HIV

Clinical Stage 1
Asymptomatic Persistent generalized lumphadenopathy
Clinical Stage 2
Unexplained moderate weight loss (<10% of presumed or measured body weight)' Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis) Herpes zoster Angular Cheilitis Recurrent oral ulceration Papular pruritic eruptions Seborrhoeic dermatitis Fungal nail infections
Clinical Stage 3
Unexplained 2 servere weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhoea for longer than one onth Unexplained persistent fever (above 37.5OC intermittent or constant for longer than one month) Persistent oral candidiais Oral hairy leukoplakia Pulmonary tuberculosis Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia) Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis Unexplained anaemia (<8 g/dl), neutropenia (<0.5 x 109/litre) and or chronic thrombocytopenia (<50 x 109/litre3)
Clinical stage 4[3]
HIV wasting syndrome Pneumocystis pneumonia Recurrent severe bacterial pneumonia Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site) Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs) Extrapulmonary tuberculosis Kaposi sarcoma Cytomegalovirus infection (retinitis or infection of other organs) Central nervous system toxoplasmosis HIV encephalopathy Extrapulmonary cryptococcosis including menigitis Disseminated mycosis (extrapulmonary histoplasmosis, coccidiomycosis) Recurrent septicaemia (including non-typhoidal salmonella) Lymphoma (cerebral or B cell non Hodgkin) Invasive cervical carcinoma Atypical disseminated leishmaniasis Symptomatic HIV-associated nephropathy or symptomatic HIV – associated cardiomyopathy
1 Assessment of body weight in pregnant women needs to consider expected weight gain of pregnancy. 2 Unexplained refers to where the condition is not explained by other conditions. 3 Some additional specific conditions can also be included in regional classifications (e.g. reactivation of American trypanosomiasis (meningoencephalitis and / or myocarditis) in Americas region, Penicillionsis in Aisa)

Anti Retro Viral Therapy.

Pre ART care is attributed to the period before ART initiation , since they are found to have a better immune and clinical staging Patients who do not require ART should be counselled for a good healthy living habits and environment and should be linked with health providers and facility centres[10] .

1. Baseline screening of CD4.
2. Baseline lab assessment that includes basic bloods and Urine analysis.
3. Yearly PAP smear testing for women
4. HBsAg and HCV screening for IDUs.

The universal goals of ART are to improve the quality of life among PLHIV by reducing the HIV related morbidity and mortality by providing maximal and durable suppression of viral load and by restoring the immune function .Screening for TB is done in every visit,

All the patients should be assessed clinically by Clinical the Stage of HIV disease and the patient's past illness and the current HIV related illness and also the need for ART and OIs prophylaxis should be determined .Other co-existing medical

conditions should be sought after and that may influence the choice of regimen.

HIV infected Adults & Adolescents (Including pregnant women)	
Clinical Stage I and II	Start ART if CD4 < 350 cells/mm3
Clinical Stage III and IV	Start ART irrespective of CD4 count
For HIV and TB co-infected patients	
Patients with HIV and TB co-infection (Pulmonary/ Extra-Pulmonary)	Start ART irrespective of CD4 count and type of tuberculosis (Start ATT first, initiate ART as early as possible between 2 weeks to 2 months when TB treatment is tolerated)
For HIV and Hepatitis B and C co-infected patients	
HIV and HBV / HCV co-infection – without any evidence of chronic active Hepatitis	Start ART if CD4 < 350 cells/mm3
HIV and HBV / HCV co-infection – With documented evidence of chronic active Hepatitis	Start ART irrespective of CD4 count

Table 6: CD4 monitoring and follow-up schedule	
CD4 Count	Follow up
CD4 of any value and on ART	Every 6 months
Between 350 and 500 and not on ART	Repeat at 3 months
>500 and not on ART	Repeat at 6 months
Note: If the CD4 count is between 350 to 400 cells/mm3 and the patient is not on ART; repeat CD4 assessment after 4 weeks and consider treatment in asymptomatic patients. *See Table 13 for more details p19.*	

HAART (Highly Active Anti- retroviral Therapy)

HAART is defined when 2 or more NRTIs are combined with a single NNRTI or a Single PI or when a single NRTI is combined with a PI and a NNRTI or when a triple NRTI is combined with an Abacavir back bone. The Standard drugs as per the NACO guidelines are

First-line regimen		Second-line regimen	
		NRTI component	PI component
Standard Regimens	AZT + 3TC + NVP AZT + 3TC + EFV TDF+ 3TC + NVP	Choices: 1st- TDF + ABC or 2nd- ddI + ABC or 3rd- TDF + AZT (± 3TC)(ii)	Choices: 1st - ATV/r 2nd- LPV/r 3rd- SQV/r 4th- IND/r 5th- NLF
Special circumstances	D4T + 3TC + NVP TDF + 3TC + NVP TDF + 3TC + EFV	Choices: 1st- ddI/ABC 2nd- ddI/AZT (± 3TC)(ii)	

The HAART drugs used in the NACO programme and that are available in India are

Nucleoside reverse transcriptase inhibitors (NRTI)	Non-nucleoside reverse transcriptase inhibitors (NNRTI)	Protease inhibitors (PI)
Zidovudine (AZT/ZDV)*	Nevirapine* (NVP)	Saquinavir* (SQV)
Stavudine (d4T)*	Efavirenz*(EFV)	Ritonavir* (RTV)
Lamivudine (3TC)*	Delavirdine (DLV)	Nelfinavir* (NFV)
Didanosine (ddI)*	Fusion inhibitors (FI)	Amprenavir (APV)
Zalcitabine (ddC)*	Enfuviritide (T-20)	Indinavir* (INV)
Abacavir (ABC)*	Integrase Inhibitors	Lopinavir/Ritonavir (LPV)*
Emtricitabine (FTC)	Raltegravir	Foseamprenavir (FPV)
(NtRTI)	CCR5 Entry Inhibitor	Atazanavir (ATV)*
Tenofavir (TDF)*	Maraviroc	Tipranavir (TPV)

** Available in India*

Mechanism of Action of ART Drugs

NRTIs are analogues of naturally occurring deoxynucleotides, thymidine, adenosine, guanosine, cytosine and cytidine. All NRTIs are converted into triphosphate forms by intracellular phosphorylation. The main mechanism by which the NRTI inhibits viral replication is by due to its lack of 3 hydroxyl group on the deoxyribose moiety that prevents the further addition of nucleotides to the grouping DNA chain.

In contrast the non nucleoside reverse transcriptase inhibits binding to HIV reverse transcriptase, and competitively inhibits the enzyme and thereby prevents the normal movement of protein domain required for DNA synthesis[50].

The ART drugs produce a lot of side effects soon after initiation of the ART and are divided into short time (2-4 weeks), medium time (2-6 months),and long term (6-12) months side effects depending on the time of onset of the side effect .The haematological side effects which are part of the study are usually a short time to medium time side effect and very rarely happens after the first 6 month[51].

HEMATOLOGICAL MANIFESTATIONS IN HIV

HIV - 1 Infection cause an inherent suppression of the haematopoiesis and the hematopoietic aberrations that are encountered are not only associated with an increase morbidity but also hamper the efforts towards controlling the primary viral infections and the associated opportunistic infections .

These lead to an interruption or reduction of the medication doses which in turn might lead to the proliferation of the drug resistant organisms and also to the progression of infections. Cytopenias in a HIV infected patients can affect all the cell lineages individually or can present as a Pancytopenia .

It could be due to an ineffective haematopoiesis, due to a direct effect on the stem cells by the virus, or due to various alterations in the hematopoietic growth factors and cytokines, or more commonly due to the marrow toxic effect of the ART drugs or due to the invasion of the marrow of by the virus ,opportunistic infections and tumour cells and lastly by some auto immune destruction of the hematopoietic elements [52].

Anaemia in HIV.

It is the frequent Cytopenias seen with HIV and the severity of anaemia has a positive correlation with the clinical stage of the HIV. The

incidence of mild anaemia (a haemoglobin levels of <11 gm%) is about 13 %in patients receiving HAART and the incidence of Severe anaemia (<9 gm %) has been reported as 5 %. The incidence of anaemia increased with the presence of an opportunistic infections and was almost 37 % whereas it was 3 % in those who did not have any documented OIs.

Impaired erythropoiesis has been identified as the foremost cause for the anaemia with normocytic and normochromic anaemia being the most common documented type. There has been macrocytosis documented mostly with the use of Zidovudine (AZT) and there has been a typical decrease in the serum iron with decreases in TIBC as seen in chronic diseases. Serum Ferritin have been observed to be in the higher limits which parallels with the severity of HIV infection[53,54] .

The Vitamin B 12 levels have also been found to be low in HIV -1 infected patients ,but they do not manifest other manifestations of B- 12 deficiency. It has been hypothesised that the Virus interferes with the transport of the vitamin rather than the absorption as supplementation has not proved to be of any benefit . But with the available limited evidence of the role of cobalamin in hindering the attachment of the HIV-1 to CD4 cell the supplementation of the vitamin might even prove useful in patients who have sub therapeutic levels of the vitamin[55-58] .

The incidence of coomb's positive anaemia is staggeringly high among HIV infected individuals with the percentages reaching 85 % in those with AIDS and 44 % in non AIDS defining conditions and only 1 % in non HIV individuals. This has been attributed to the presence of Auto anti bodies to the U and I blood antigens on the erythrocytes and also to other non specific antigens on the surface of the erythrocytes. It is due to the non-specific binding of anti- antibodies and the deposition of specific complexes onto the surface of the erythrocytes .Haemolysis subsequent to these auto antibodies mediated destruction has been found to surprisingly rare [59] .

Paraprotienimias as part of the infection in HIV patients also contribute to the anaemia. This is mostly polyclonal rather than monoclonal and it has been demonstrated by the rouleax formation in the peripheral smear and the co migration of the paraprotiens in electrophoresis. It has also been documented that these Paraprotenimias do not cause other Cytopenias in HIV patients [59].

Anaemia in HIV patients have been documented as independent risk factor in the morbidity and mortality of the patients. A 1 % change (decrease) in the levels of Haemoglobin was shown to correlate with a 50 % change in the Hazard ratio. Thus the haemoglobin levels are not only markers of severity of the infection but also an important prognostic

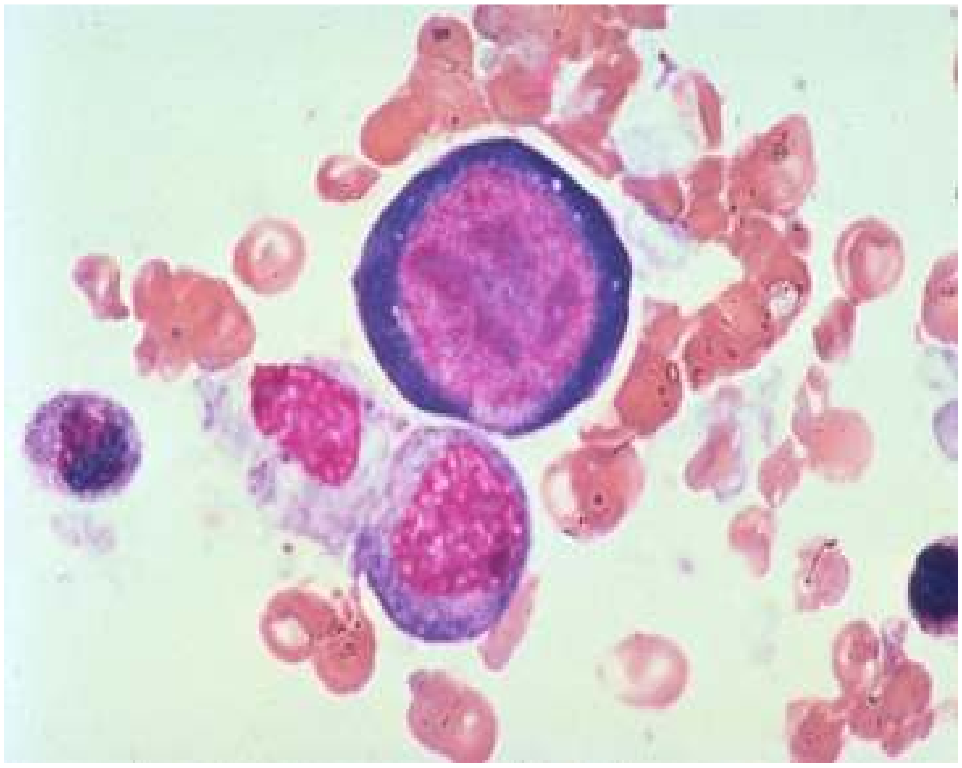
indicator. Recovery from anaemia has been associated with improved survival outcomes.

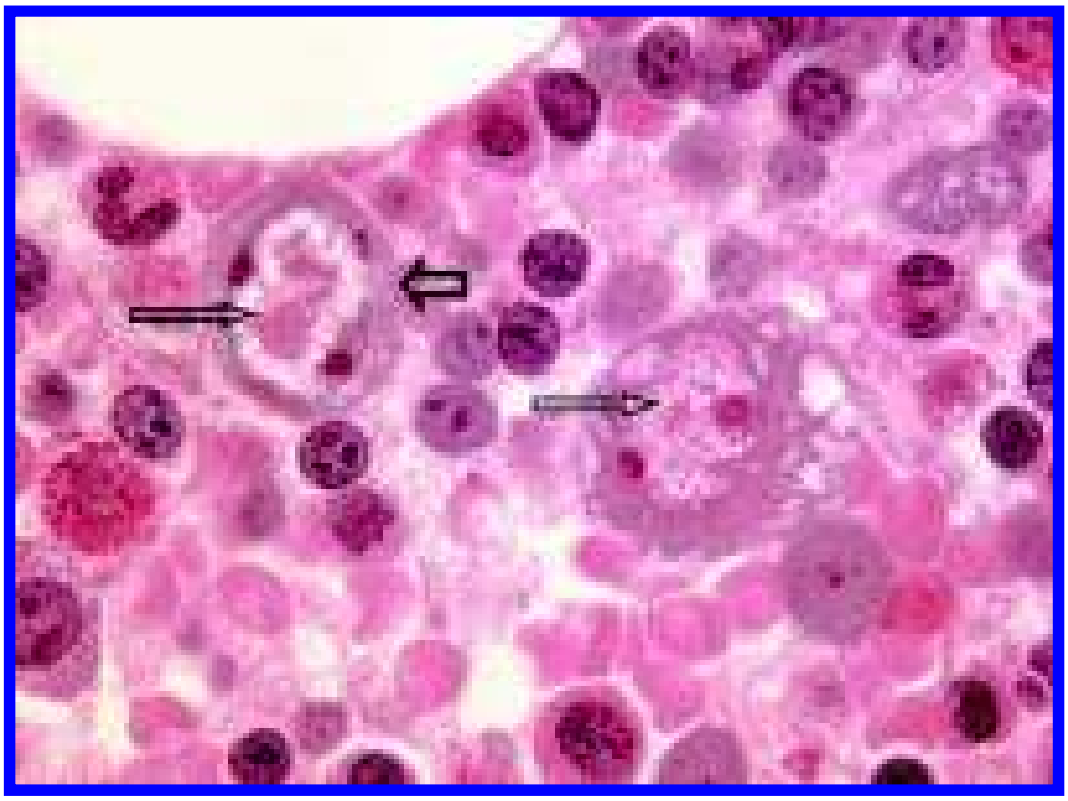
Obirikorang & Yeboah[60] and Curkendall et al.[61] had observed the correlation that a CD4 count of <200 cells was significantly associated with a severe grade of anaemia which affected the survival rate and lead to a rapid down hill disease progression.

Belperio & Rhew[62]. and Odunukwe et al[63] observed that the role of HAART IN suppressing the viral replication and in turn the viral load and reported the concomitant rise of Hb along with CD4 count rise.

Some common causes of anaemia in HIV individuals are the opportunistic infections which infiltrate the bone marrow like the Mycobacterium avium intracellulare,fungal infections like Histoplasmosis and malignancies like lymphomas.

Parvo viral B19 infections which has become persistent in that individual are some causes of intractable causes of anaemia .The peculiarity of this infection is to selectively infect the replicating Erythroid progenitor cells resulting in the decrease in the red blood cell mass and Erythroid hypoplasia.





The above picture shows the Erythroblasts which contains viral inclusions (thin arrows) at different stages of development. The giant proerythroblast on the left (thick arrow) demonstrates chromatin condensation at the periphery of the nucleus and a central viral inclusion

An intact immune system is needed to mount an action against this virus and immunocompromised patients will find it very difficult to clear this infection. This infection can be demonstrated by the presence of giant pronormoblasts.

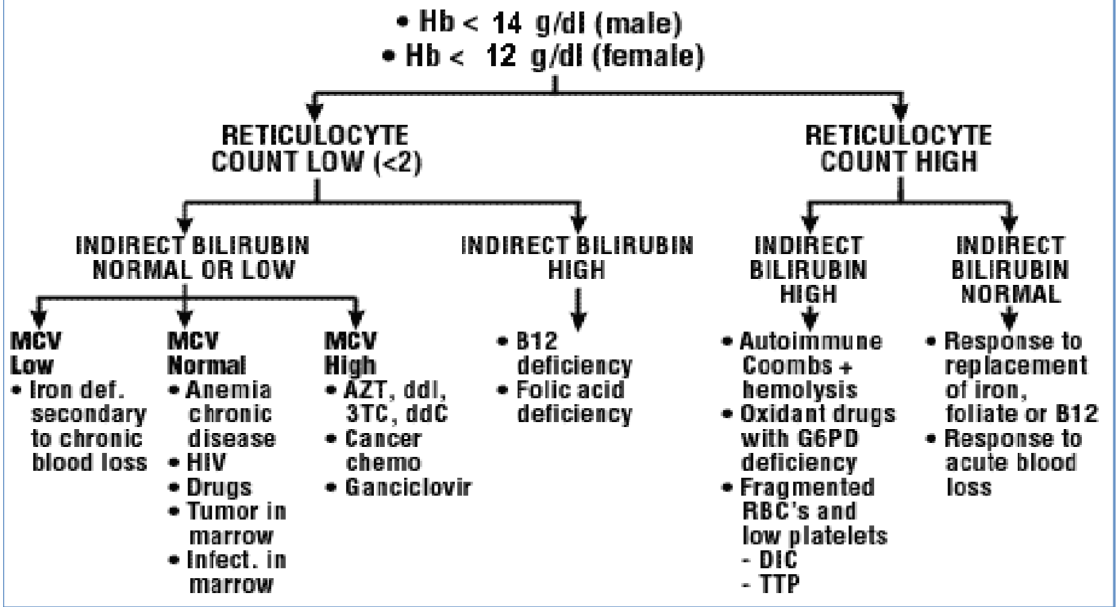
Iron deficiency has been documented in HIV patients and this may be due to chronic blood loss states which happens in Kaposi's sarcoma of the gut ,CMV colitis and in lymphomas of the gut . Folate deficiency has also been documented. These deficiencies can be corrected using appropriate supplementation of the nutrients.

Anaemia commonly occurs following the use of AZT and it manifests as an early side effect (as early as 15 days).The characteristic features are that the reticulocyte counts are depressed and this may be the first sign of bone marrow toxicity. Bone marrow examinations have revealed the complete absence or decrease of red cell precursors .

The myelosuppression cause by AZT is a transient one and is reversed by using lower doses or discontinuing the drug.It has also been

found that the erythropoietin levels are commonly elevated (> 500 IU/L) which suggests that the Erythroid hypoplasia is more due to the effect on the Erythroid stem cells rather than an interference in erythropoietin production. Recombinant Epoeitin has been to be an useful tool in treating patients without marked elevation of Erythropoietin levels. An associated macrocytosis has also been found with AZT use with a mean elevation of mean corpuscular volume (MCV) by about 25 to 30 units commonly and is not necessarily associated with anaemia (A Macrocytic normochromic pattern has been observed).This increase in MCV has been found to start happening within 6 weeks of initiation and are most prominent after about 16 to 24 weeks [64-66].

FIGURE 3: SIMPLIFIED EVALUATION OF ANEMIA IN THE HIV-INFECTED PATIENT



Neutropenia

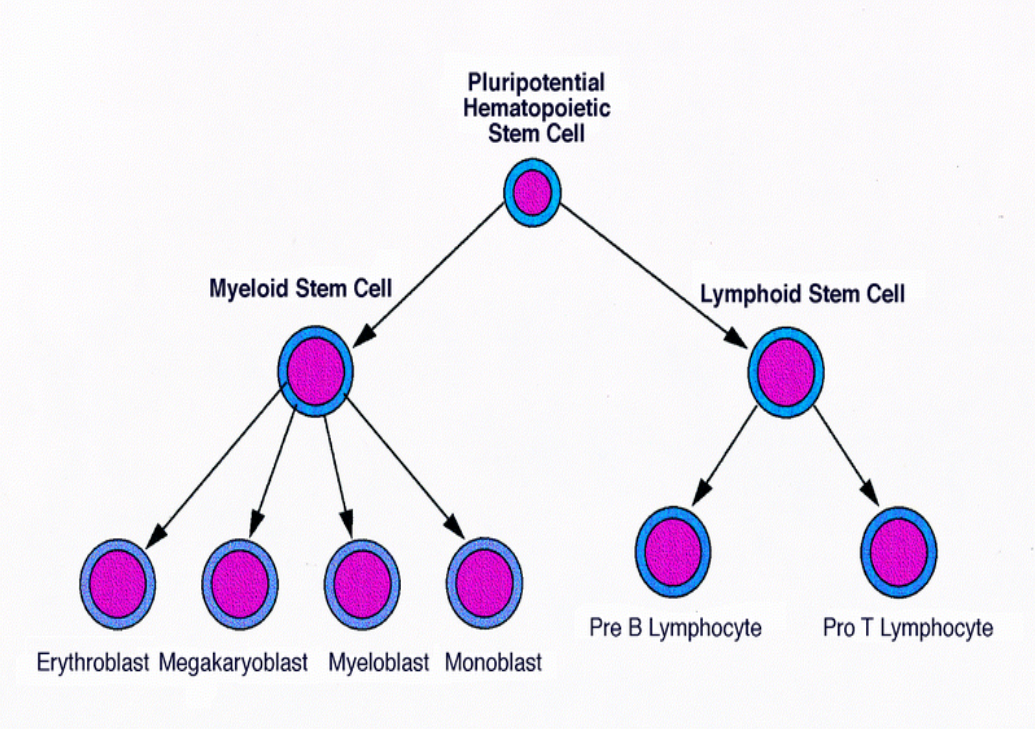
Granulocytopenia is seen to occur concomitantly with anaemia . The prevelance of neutropenia in HIV infected patients is about 30 % and the peripheral smear of those patients reveal a variable decrease in the neutrophils ,lymphocytes, and monocytes with the classical hypolobulation of the neutrophis which is commonly seen with a left shift. Vacuolization of the blood monocyte are also frequently observed .

The cause of leucopoenia has been attributed to the impairment of the myelopoiesis and the myelotoxic drugs are the frontrunners as the causative agents in almost 80% of patients. Some patients have been found to have some circulating antibodies on their cell surface .The HIV infection has both direct and indirect effect on the granulocyte numbers which causes a steady decline in the oxidative and chemotactive capabilities.

There also occurs a steady increase in the apoptosis rate. A qualitative defect in the neutrophils have been observed in the HIV Patient and these defects make the neutrophils less pliable with respect to diapedesis and these defects tend to correct with the administration of Colony stimulating factors (Granulocyte -Monocyte colony stimulating factors-GMCSF) and also with the initiation of HAART .

There has been even reports of deficiencies in the production of G-CSF in response to neutropenia although the response to other febrile episodes appears normal. About a third of patients also have anti - neutrophils antibodies but this does not have any correlation with the severity of neutropenia.

Granulocytopenia in HIV patients significantly increases the risk of severe bacterial infections [67-71] .



Drugs associated with Neutropenia.

The drugs that are used in the setting of HIV that are frequently associated with neutropenia are Ganciclovir-40%, 2nd week, Zidovudine, Trimethoprim-sulfamethoxazole, Pentamidine ,Rifabutin and Dapsone.

Thrombocytopenia in HIV.

Thrombocytopenia in HIV is unique since it can occur at any stage of HIV disease ,independententely of other Cytopenias and its severity does not parallel with that of the HIV disease .The incidence of thrombocytopenia is about 60 % in all stages of HIV irrespective of the risk groups and among these about 16-40 % may present with severe thrombocytopenia (< 50,000) and may manifest bleeding manifestations .

Thrombocytopenia is neither a prognostic indicator nor plays a significant role in the morbidity outcomes. The cause of thrombocytopenia is either immune destruction or decrease production .In earlier stages of disease it has been found that immune destruction plays a predominant role whereas decrease production takes predominance in the late stages.

Majority of HIV patients have antibody coated immune complexes on the surface of the platelets .These are mostly non specific complexes which are seen .More specific complexes are seen when there are occurs

a molecular similarity existing between GP120/160 of HIV -1 virus and the GPIIB/IIIA of the platelets and these complexes coat the surface with much vigor.

This process causes more destruction of the platelets and the life span of those cells decrease by about 2/3 rd's after the antibodies have been detected and also it has been found that the presence of antibodies. Besides the immune destruction other infections and fevers can also play a significant role in decreasing the life span of the circulating platelets.

The other mechanisms of decreased platelet count are due to Splenomegaly which causes sequestration of platelets, and non immune destruction of platelets as occurs in the hemolytic uremic syndrome and thrombotic thrombocytopenic purpura which is found to occur more frequently in HIV infected patients [72,73] .

Even though the megakaryopoiesis is stimulated there is a steady decrease in the production capability of the megakaryocytes which counters the peripheral destruction .The precursors demonstrate a significant raise in apoptosis when it is compared with non HIV patients and Various studies have shown an inverse relationship with circulating platelets.

Other mechanisms are that the HIV-1 might directly target the megakaryocytes and thereby suppressing the platelet production and the numbers may be low and there may occur qualitative and morphologic changes amongst the megakaryocytes.

The virus also alter the antigens on the surface of the megakaryocytes and makes them as sitting ducks for antiplatelet antibodies .There are various dysregulations that are found in the growth factors and the cytokines in HIV patients which can alter the platelet production reducing it numbers.

HIV associated Thrombocytopenia is a difficult situation to treat with as there are no published randomized trials on the treatment of thrombocytopenia. There have been anecdotal reports of spontaneous remission .Sudden increase in the platelet counts are associated with a deterioration of the immune system and that might be a forerunner for the AIDS complex . In patients with decrease in the counts there is no necessary to treat as the bleeding in those patients seldom occur despite a low platelet count[74-76] .

Some time AZT treatment might result in the increase of the platelet counts in 30 %of patients and this results can be seen within 8-12 weeks after initiation of AZT based HAART. Dapsone has also shown a similar effect on the Platelet counts and the mechanism by which it

happens has been postulated due to a decrease in the phagocyte mediated destruction of the *cells*.

Corticosteroids have also been tried to increase the platelet counts with varying results .There are other modalities which have been tried to improve the platelet counts and they are anabolic steroids and vincristine .

The response rate for vincristine has been around 10 % and high dose ascorbic acid has also been to show better results when given in small quantities over several months. Interferon alpha has also been tried mostly in AZT resistant thrombocytopenia and the response has been good in almost 70 % of patients and the mechanism by which Interferon acts has been postulated as by increasing the IL - 6 levels which has a trophic effect on platelets.

Gamma globulin infusion has shown to cause rapid rise in platelet counts and the median time duration has been in the order of 3-4 weeks rather than months in other modalities even thought the sustained remission is hard to maintain. A similar response (75%) is seen with the administration of Anti-D antibody .The last two modalities the gamma globulin and the anti-D immunoglobulin saturates the complement receptors in the reticuloendothelial system thereby hindering the platelet destruction .

When these modalities have failed splenectomy has been tried with good results and these interventions have also transiently raised the CD4 counts but they continue to be at a greater risk for fulminant opportunistic infections. Another alternative to splenectomy has been the low dose splenic irradiation which has in uncontrolled studies showed an acute response of 70% and sustained responses observed in over 40% of HIV-1 patients[77,78].

Causes Of Cytopenias in a HIV patient .

The process of hematopoiesis is both an inducible and a constitutive one. The constitutive process is under the control of the colony simulating factors whereas the inducible process is under the control of cytokines and interleukins that influence hematopoiesis in situations of altered demand.

The cytokines have the origin from the marrow blasts , endothelial cells,T Cells, and monocytes. The alterations in cytokines and the growth factors because of the HIV infection contribute to the hematopoietic abnormalities. Other contributory factors to the Cytopenias are opportunistic infections and neoplasm's and Myelosuppressive medications.

Cytopenias due to Tumor, infection and Medications.

Lymphomas, Kaposi's sarcomas and Squamous Cell carcinoma are common to HIV and the bone marrow is found to be involved in one third of those with lymphomas it is the non cleaved cell type which is frequent .Other causes are the Myelosuppressive medications which are used in HIV as it has been found that when these drugs are used in situations which have altered hematopoietic potential they potentiate the Myelosuppressive effect which are not commonly seen in non HIV patients.

HIV and Various Colony stimulating factors.

The important factors that are used in HIV are the Granulocyte , Monocyte, granulocyte/monocyte colony stimulating factors and the IL-3 ,stem cell factors and erythropoietin.

These glycoprotein's control the hematopoietic cells in the cell maturation cycle from start to terminal maturation and the major role of the cytokines is the suppression of the apoptosis .

The B and T lymphocytes and the Natural killer cells are the prime contributors of these colony stimulating factors. These cells are also the primary targets for the HIV as well and when these cells are infected they

progressively decrease in numbers and thereby decreasing the colony stimulating factors.

The production of these factors increases during early infection and this attributes to the hyperrcellularity of the bone marrow seen at the earlier stages. The levels of IL -1 , TNF alpha and interferon Gamma are low during the early stages as well.

As the disease advances the levels of the cytokines increase considerably and these in turn start to inhibit hematopoiesis rather than stimulating it and also the increasing cytokines alters the receptors on the target cells to make them less responsive to the stimulating growth factors.

Moreover inhibiting cytokines like the (TGF -Beta) are also found to be in increasing amounts and they also in turn inhibit hematopoiesis.

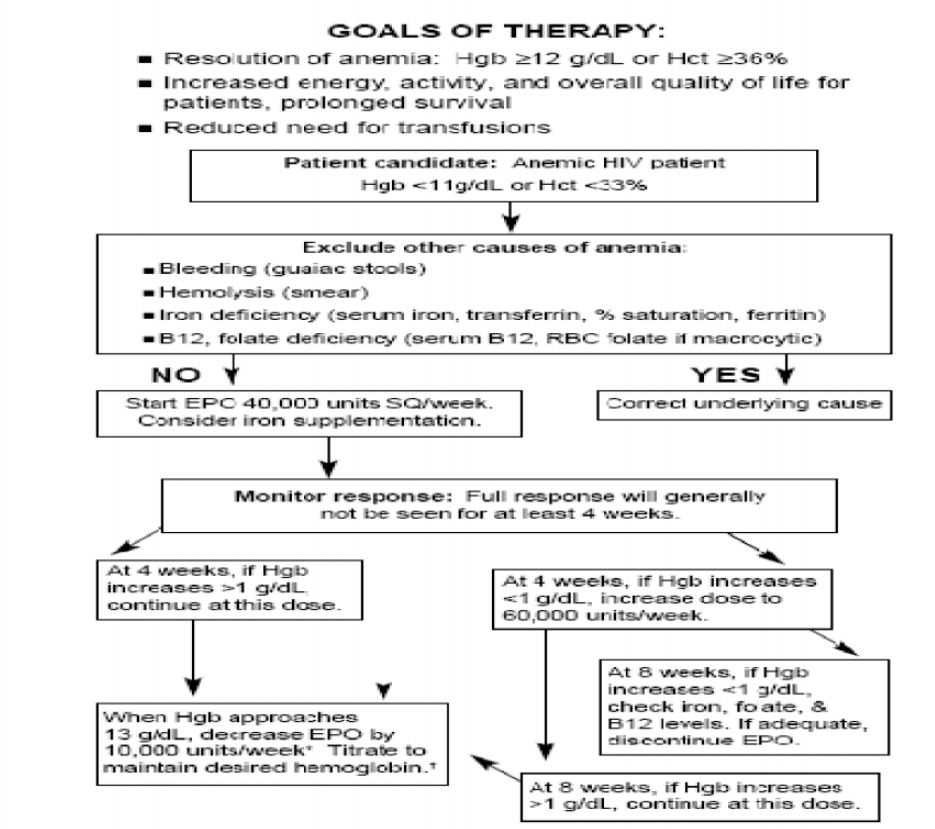
These negative factors of Hematopoiesis are produced by the HIV infection itself, with the Tat Protein being released from the monocytes stimulates the release of TNF -alpha, and the Nef protein that may be released from the infected cells induces the production of IL-6.

All these are considered in the usage of the colony stimulating factors in treatment of the Cytopenias with the exogenous administration

of these factors often leads to the amelioration of the effects on the Hematopoiesis.[79-83]

Role of Erythropoietin.

1. Maintain use of growth factors for at least several mos.
2. Taper dose slowly as tolerated to maintain Hb 11-12
3. Maintain adequate iron stores with iron supplementation.
1. 4.Inadequate iron stores most common reason for non-response to growth factors.
2. 5.Erythropoietin corrects anemia in HIV/HCV-co infected patients treated with IFN/RBV, including those taking AZT
1. Complete correction of anemia with erythropoietin in patients with chronic kidney disease increases risk of CV events and death.
2. Relevance to HIV unknown, but suggests that correction of Hgb to >12.0 g/dL should not be goal of treatment.



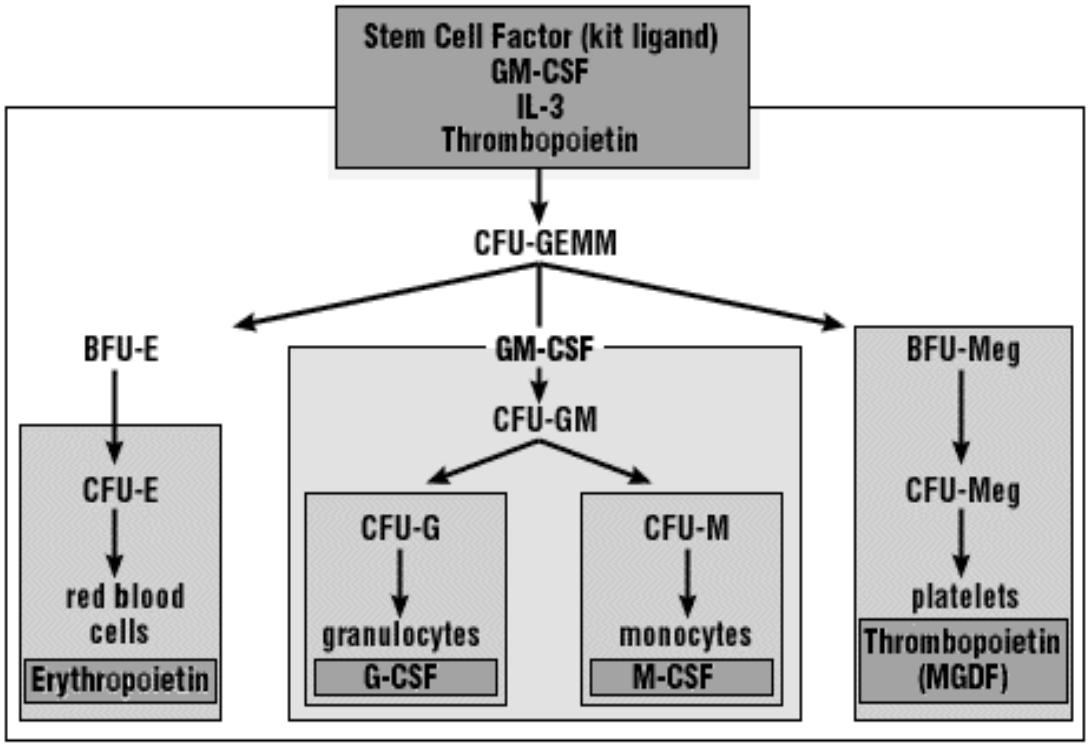
Role of G CSF

HIV-1 patients who have been initiated on a HAART regimen and are exhibiting Cytopenias, the G-CSF administration substantially increases the concentration of CD4 + and CD8 + lymphocytes and the NK cells. High dose administration causes modest elevation of monocytes and also causes absolute increase in the neutrophil count. When GM -CSF is given along with the concomitant ART the antiviral effects are also increased due to the raise in the active drug concentration within the monocytes and this effect has been shown only with GM -CSF.

Stem cell Factor (SCF,Kit Ligand)

Marrow Stromal cells produces a growth factor which acts on the myeloid ,lymphoid, and mast cell lineage and it acts in hand in hand with the other trophic factors. The SCF levels decreases as HIV advances and this correlates with the survival as well and when it is given to HIV-1 patients haematopoiesis is promoted considerably in a dose dependent fashion without any alteration of the HIV expression.

FIGURE 2: HEMATOPOIETIC GROWTH FACTORS



Interleukin -3

It is another factor that is produced by the activated T cells and has also direct effects on all other nucleated cells .Administration causes an increase in circulating granulocytes ,erythrocytes, and platelets. When the T cell numbers are decreasing in late stages it causes reduction in endogenous IL-3 which may contribute to myelosuppression and it has also been documented that suboptimal levels of IL-3 has a synergistic effect with other factors to counter the myelosuppression associated with HIV infections.

The mechanism that has been postulated is the prevention of apoptosis in the progenitor cells that is commonly seen in advanced HIV infection with reduced levels of growth factors.

Interleukin -1

It is an acute phase reactant produced by the monocyte ,dendritic cells,lymphocytes,NK cells, fibroblasts and it induces the secretion of hematopoietic factors like G CSG, GMCSF and M CSF and also Il-6.If IL-1 secretion is continuously secreted it can lead to the increase production of TNF alpha that in turn suppresses the hematopoietic activity and it has also been found that the IL-1 increases with the progression of the HIV infection.

Interleukin - 2

It is a cytokine which acts as a growth factor for the lymphocytes and it induces the synthesis of Interferon Gamma which decrease haematopoiesis and also increases the HIV -1 replication .

Interleukin-6

This cytokine is produced by the B and T cells, fibroblasts, eosinophils ,and vascular endothelial cells and it has also been directly and indirectly up regulated by the HIV-1 Tat Protein. IL -6 and IL - 3 acts synergistically to enhance haematopoiesis and IL-6 also up regulate the HIV-1 production by the infected cells and the enhanced T cell proliferation expands the HIV Infected cell pool .

Interferons

This cytokine level increase with the progression of HIV infection and interferon -alpha inhibits marrow progenitor cells and this is by a supplementary effect by other cytokines which are stimulated directly by the interferons. Interferon -gamma up regulates the CXCR4 receptors on the bone marrow which is present of the maturing megakaryocytes and platelets and these being the co-receptors for HIV-1 causes a decrease in platelet counts with a rise in HIV viral load as occurring in late HIV infection.

Tumor Necrosis Factor -Alpha.

This cytokine is produced by the monocyte and macrophages and the Tat protein of HIV-1 infection activates the genes capable of producing TNF alpha. This cytokine is a both positive and negative regulator of haematopoiesis.

Transforming growth factor - Beta.

This is a stimulatory agent particularly for the fibroblasts and is an inhibitor for hematopoietic proliferation and a reversible suppressive effect on the marrow progenitor cells. The levels of the cytokine also increases with HIV -1 infection and it is also increased by the Tat Protein of HIV and this also enhances the HIV -1 expression in infected cells .

Coagulation abnormities associated with HIV.

These abnormalities have been commonly noticed in HIV-1 infection and in most of the situations and it has been attributed to the antiphospholipid antibodies which binds to cardiolipin,anionic phospholipids,beta-2 Glycoprotein -1 which predominantly inhibits coagulation and platelet aggregation. These antibodies are due to the abnormal immune responses as part of the HIV-1 infection and these titres vary with stage of the HIV infection and with the presence of opportunistic infections. It has been observed that the thromboembolic

manifestations that occur as a part of these antibodies are less in HIV-1 infected patients when compared with general population.

It has also been observed that the protein -C and Protein- S levels are also significantly less in HIV-1 infected patients and these correlate with CD4 counts. The Protein S has been postulated to bind to the C4 binding protein, and a subsequent increase in the protein-S levels with chronic HIV infection results in a more compact binding of Protein S and thereby less amounts are available to prevent aberrant thrombotic even. There are also anti-Protein S antibodies which are seen in HIV-1 infections which also contributes the lower levels .The levels of Heparin Co factor II is found to be lower in PLHIV and this level further decreases in advanced HIV infection.

Several OIs have been associated with a Pro thrombotic State in PLHIV by mechanisms that restrict the expressions of Pro - Coagulant phospholipids to sites of vascular injury. The infections like CMV and HSV 1 & 2 have the ability to convert the vascular endothelial cells to a pro coagulative phenotype by altering the surface phospholipids which are active in the coagulation system.

There have been documented evidences of increase in the Von willebrand factor with the increase in CD4 counts and also a decrease in the plasminogen activator levels correlates well with increasing HIV-1 levels contributing to a prothrombotic stage. The hypoalbuminemia associated with the HIV infection also contributes to fibrinolytic defects .The incidence of Thrombotic thrombocytopenic Purpura is also more in HIV infected patients and the exact mechanism of this consumptive coagulopathy has not been studied yet and plasmapharesis with exchange has been proved effective to treat these patients.

There also a hypergammaglobulinemia that has been documented and it has been due to a polyclonal B-cell activation and it is associated with inability to form antibodies when exposed to new antigens. It causes rouleax on peripheral blood smear and it also parallels' generalized lymphadenopathy. Another interesting manifestations is the Hemophagocytic Syndrome which manifests with fever, Hepatospelnomegaly, and lymphadenopathy and Pancytopenia and shows the classical cells in the peripheral smear[84-89].

Bone Marrow findings in HIV.

The bone marrow in HIV exhibits a multitude of morphologic aberrations. The typical bone marrow features are as follows.

Cellularity: Increased in 50–60%
Normal in 35–40%
Hypocellular in 5%

Dysplasia in one or more cell lines occurs in over 70%
Granulocyte > Erythrocyte > Megakaryocytic

Lymphoid aggregates occur in 20%

Fibrosis occurs in 20%

Less commonly seen: Eosinophilia
Plasma cell in Itrates

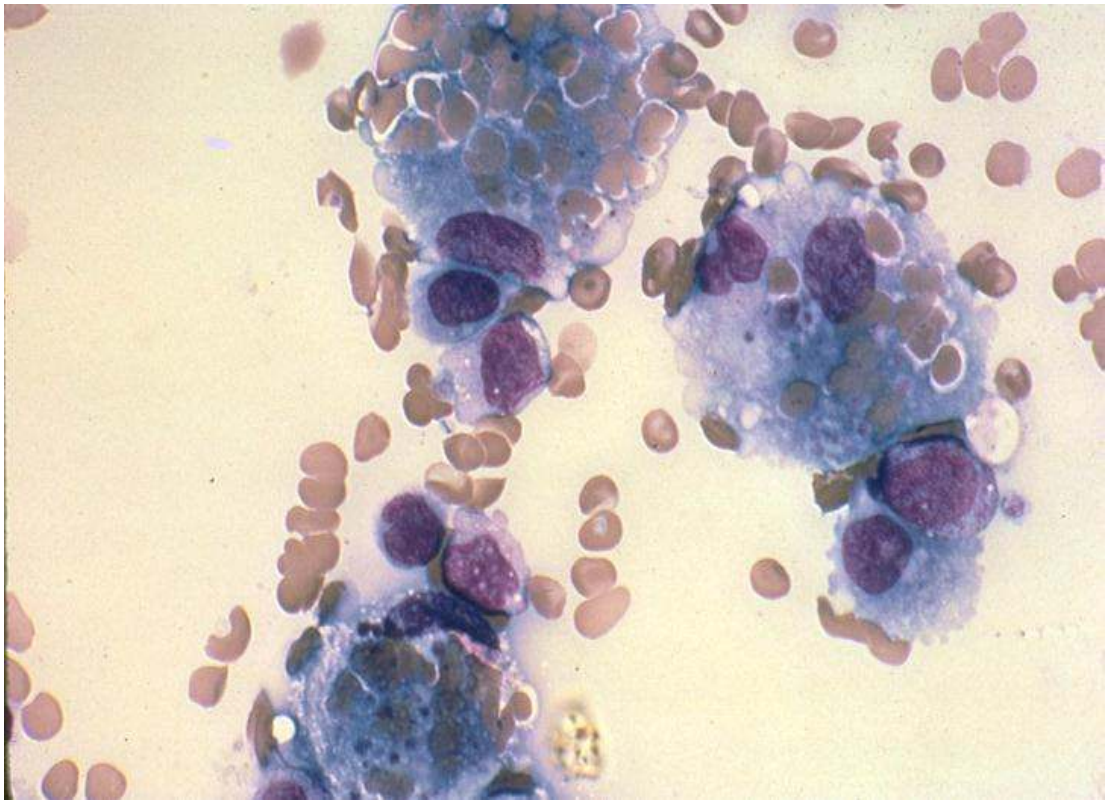
Hypercellularity of the marrow has been demonstrated as the most common finding in about a 50% of patients and is due to absolute hyperplasia of one or more non -lymphoid cell lineages.

The Myeloid Erythroid ratio tends to be normal often and the marrow cellularity has been documented to correlate with neither the peripheral blood counts nor with the stage of the HIV disease. The hypocellularity of the marrow is quite a rare manifestation found in less than 5% of patients and is seen in more advanced stages of the infection as a fore runner to the atrophy and necrosis of the marrow which is seen in end stages of HIV-1 infection.

It has been found that the dysplasia of one cell line is found in about 70% of patients and is similar to the myelodysplastic syndrome .The dysplasia of the granulocytic series with a characteristic vacuolization of the precursors is seen in both the peripheral smear and marrow and is the most common one followed by the dysplasia of the erythrocytic dysplasia in 50-60% and megakaryocytic series changes which is seen in about a third of patients and these changes increase in frequency with the presence of coexistent opportunistic infections.

Other manifestations that are noted are the iron over load in the maturing Erythroid cells and the incidence is around 60 %. The less common changes that are noted include the aggregation of lymphoid cells in about 20 % of patients and this occurs despite the presence of

peripheral lymphopenia which can happen in HIV infection.



There also occurs marrow fibrosis with advancing HIV infection and increase in marrow deposition of reticulin and this is seen with mycobacterium and fungal infection .There are also certain non specific changes which include histolytic erythrophagocytosis mild increase in the eosinophils and plasma cells[90-92].

METHODOLOGY

Title Of the Study

A study on Haematological Changes following first Line HAART (Highly Active Anti retroviral Therapy) in Adult HIV -1 Infected Patients.

Data Collection and the Source

HIV Patients admitted to Medical Units with Haematological changes after initiation of first line HAART during the study period.

HIV Patients Reviewed at the ART Centre at CMCH with Haematological changes soon(day 15,1 month,6 month) after the initiation of first line HAART during the study period

Data collection Methods

All Adult HIV patients between the time period of Aug 2013 till July 2014 Who developed Haematological Changes Post First Line HAART initiation was included in the study.

This study includes the Detailed History, Clinical Features, Peripheral Smear Study, Automated CBC counts, and Bone Marrow of the HIV patients developing Haematological complications after initiation of the HAART.

Sampling Method.

Prospective Cohort Study with Consecutive Sampling methods

Sample Size : 52 Patients.

Case Definitions

Defined as per the National Aids Control Organisation (NACO) HIV/ART guidelines (First line HAART, Anaemia < 10 gms %)

Inclusion Criteria

All adult HIV Patient who are initiated on First line HAART as per the NACO guidelines at CMCH and who develop mild to severe haematological changes (anaemia, thrombocytopenia, Pancytopenia

Exclusion Criteria

Patients on Myelosuppressive drugs (other than Cotrimoxazole)

Patients who develop IRIS after initiation of HAART.

Patients with diagnosed haematological problems (Malignancies, Hereditary disorders, anaemia of Chronic Disease)

Patients who are on treatment for both HIV and TB.

Pregnant women and children with age < 15

Patients who are on treatment for HIV and CMV.

Patients with both HIV and Hep B and Hep C infections.

Patients on Second Line HAART ,Patients with Renal Failure

Patients with HIV -2 infections and Patients with poor adherence (< 80%).

Analysis: All the Data were collected on to the Data collection sheet in an excel format and Analysis was done using SPSS statistical Software and proportions were compared using Chi -Square test of Significance .A 'p' value' of less than 0.05 was considered statistically significant.

Characteristics of the study population

52 Patients presented with anaemia and Cytopenias and were observed for a period of over 6 months .The anaemia was equally distributed among both sexes and most of them (90%) were in the reproductive age group of 20 - 49 and 92 % of them were in stage 3 or stage 4 disease when they were initiated on AZT based HAART.

CHARACTERISTICS OF THE STUDY POPULATION				
Parameter	**Male (n=26)**	**Female (n=26)**	**Total (52)**	**Percentages (100)**
Age				
0-19	0	1	1	1
20-29	2	10	12	23
30-39	11	9	20	38
40-49	10	4	14	27
>50	3	2	5	9
WHO Stage.				
Stage 2	3	1	4	8
Stage 3	9	6	15	29
Stage 4	14	19	33	63
CD4 at Baseline				
>200	13	10	23	44
50-199	12	13	25	48
<50	1	3	4	7
Type Of Anemia observed				
NORMOCYTIC NORMOCHROMIC	2	7	9	17
MICROCYTIC HYPOCHROMIC	16	5	21	40
NORMOCYTIC HYPOCHROMIC	3	7	10	19
MACROCYTIC HYPOCHROMIC	5	7	12	23

CHARACTERISTICS OF THE STUDY POPULATION				
RBC COUNTS	**Male (n=26)**	**Female (n=26)**	**Total (52)**	**Percentages (100)**
<2	13	7	20	38
2-2.9	9	12	21	40
3-3.9	4	5	9	17
>4	0	2	2	3
Hematocrit.				
<20	14	4	18	34
20-29	7	11	18	34
30-39	3	7	10	19
>40	2	4	6	11
Total Counts				
<1000	1	1	2	3
1000-1999	2	4	6	11
>4000	23	21	44	84
Platelet counts				
0.5	4	8	12	23
0.5-0.99	3	6	9	18
1-1.49	1	0	1	1
>1.5	18	12	30	58
MCV				
<70	12	0	12	23
70-79	4	5	9	17
80-89	4	10	14	27
90-99	3	6	9	17
>100	3	5	8	15
MCH.				
<25	15	11	26	50
25-30	10	10	20	38
>30	1	5	6	11

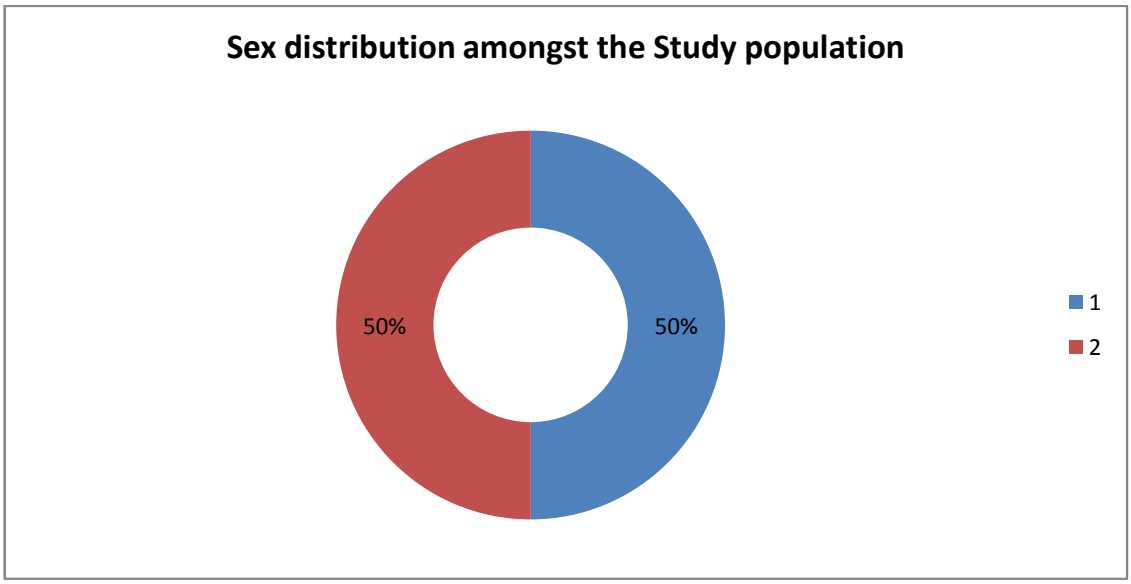
Equal number of patients were found to have a CD4 counts above and below 200. 40 % of the study population had a Microcytic Hypo chromic anaemia and the other types were found to be equally distributed. RBC counts were found to be less than 2 million in about 38 % of

patients and the Hematocrit was found to be less than 20 in about 34 % of patients.

Mild Thrombocytopenia was noted in 42 % of the population and Moderate to Severe thrombocytopenia (<50,000) was observed in 23 % of them . MCV was low in 40 % of patients and MCH was low in 50 % and this equates well with the 40 % incidence of Microcytic Hypo chromic anaemia in this study population.

2. Sex distribution amongst the Study population.

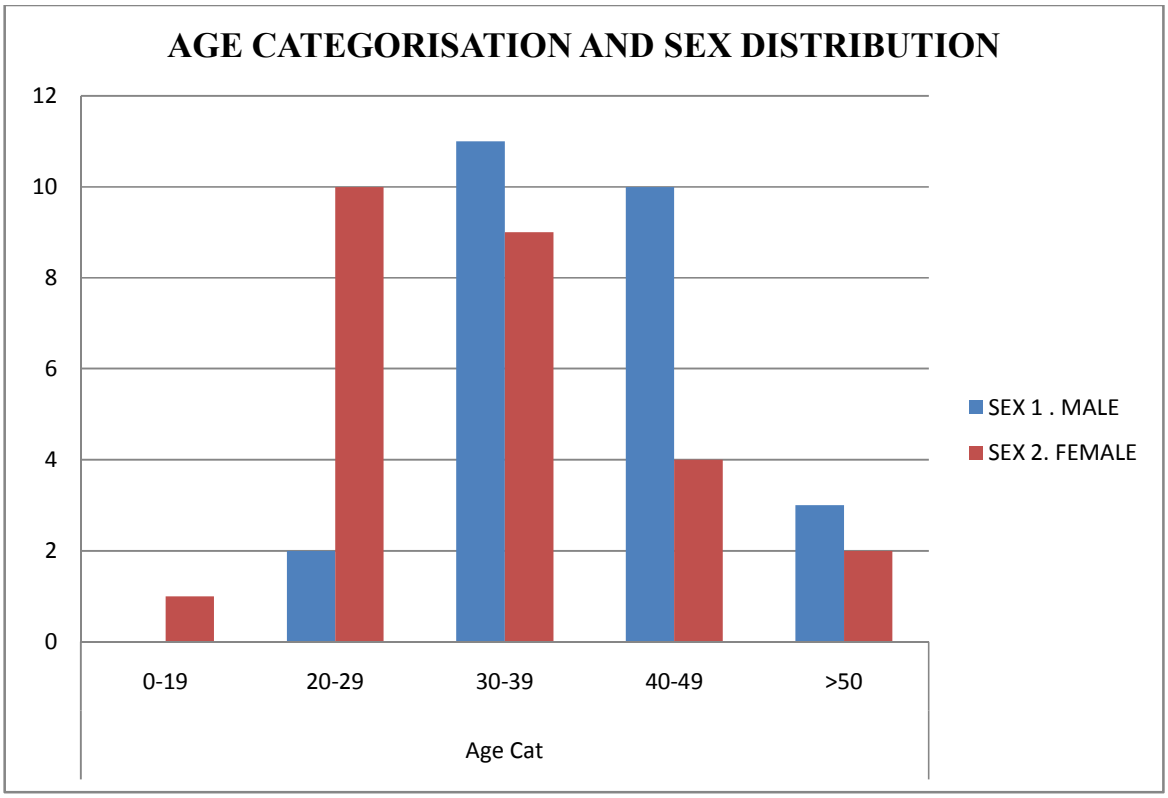
Sex	Frequency	Percent	Valid Percent	Cumulative Percent
1.Male	26	50.0	50.0	50.0
2.Female	26	50.0	50.0	100.0
Total	52	100.0	100.0	



In the study population the sex distribution was evenly distributed with both males and females being represented in a cumulative percentage of 50 % which is pictorially depicted in the above picture.

3. AGE CATEGORISATION AND SEX DISTRIBUTION

Age Categorisation	SEX		Total	Percent
	1 . MALE	2. FEMALE		
0-19	0	1	1	1.9
20-29	2	10	12	23.1
30-39	11	9	20	38.5
40-49	10	4	14	26.9
>50	3	2	5	9.6
Total	26	26	52	100.0

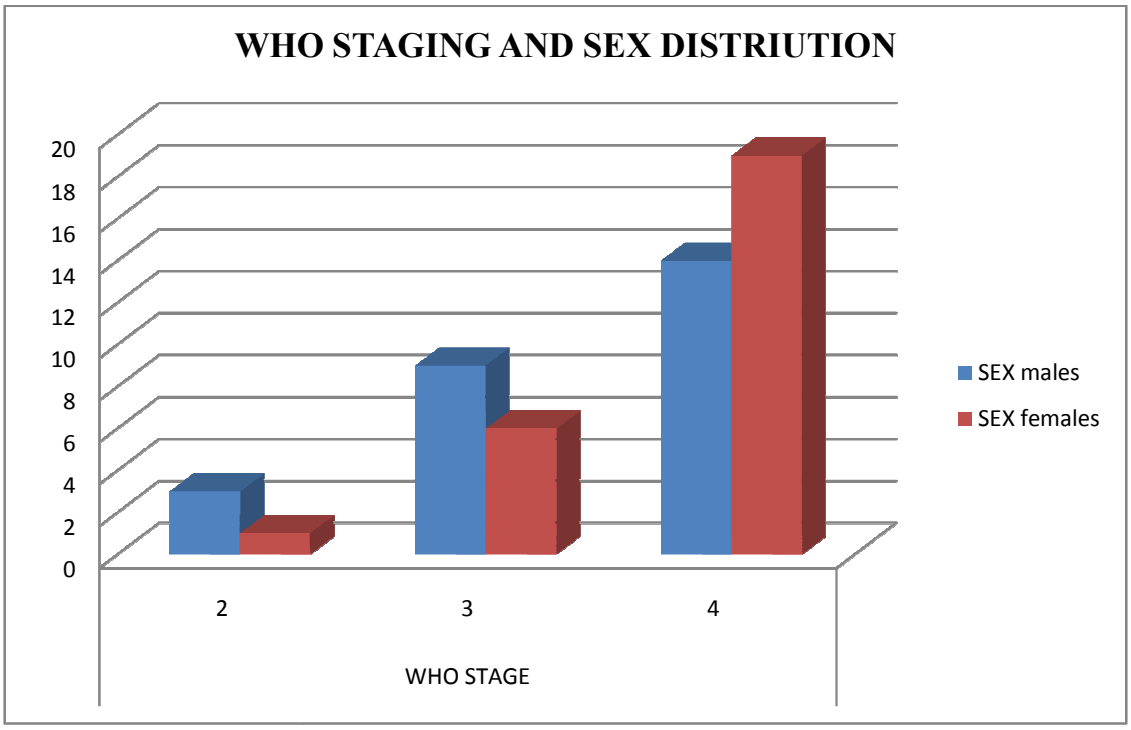


Among the study population 88% of the patients were in the reproductive age group of 20-49 with the males representing by a slight higher margin in the age group category of 30-39 and 40-49

4. WHO STAGING AND SEX DISTRIUTION

		SEX		Total	Percent
		Males	Females		
WHO STAGE	2	3	1	4	7.7
	3	9	6	15	28.8
	4	14	19	33	63.5
Total		26	26	52	100.0

P VALUE 0.308

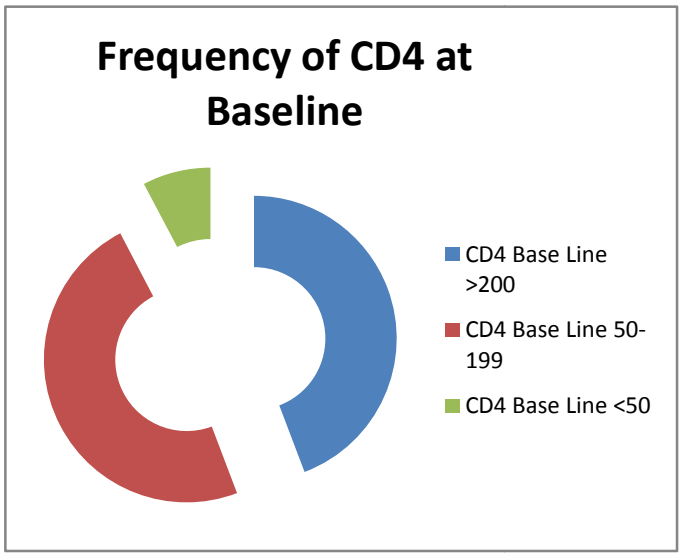


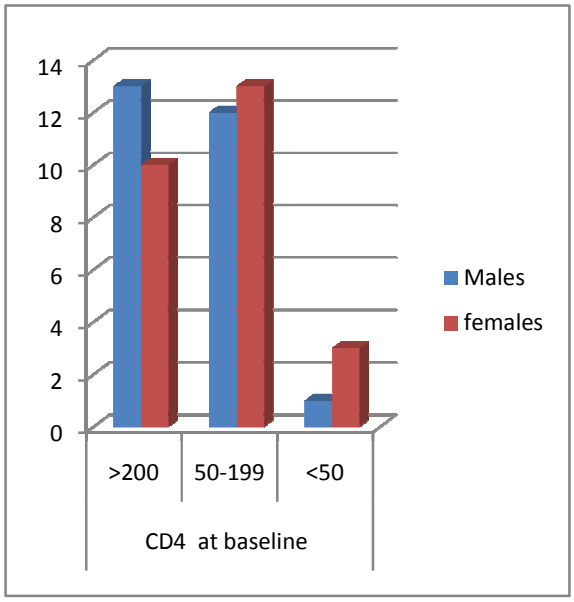
It was observed that 63.5 % of patients were in the stage 4 disease and there was no significant correlation (p- value of 0.308) between the WHO stage and the sex distribution in the study population.

5. **Frequency of Baseline CD4 counts and Sex distribution**

CD4 at baseline	Sex distribution		Total	Percent
	Males	females		
>200	13	10	23	44.2
50-199	12	13	25	48.1
<50	1	3	4	7.7
Total	26	26	52	100.0

p VALUE - 0.489



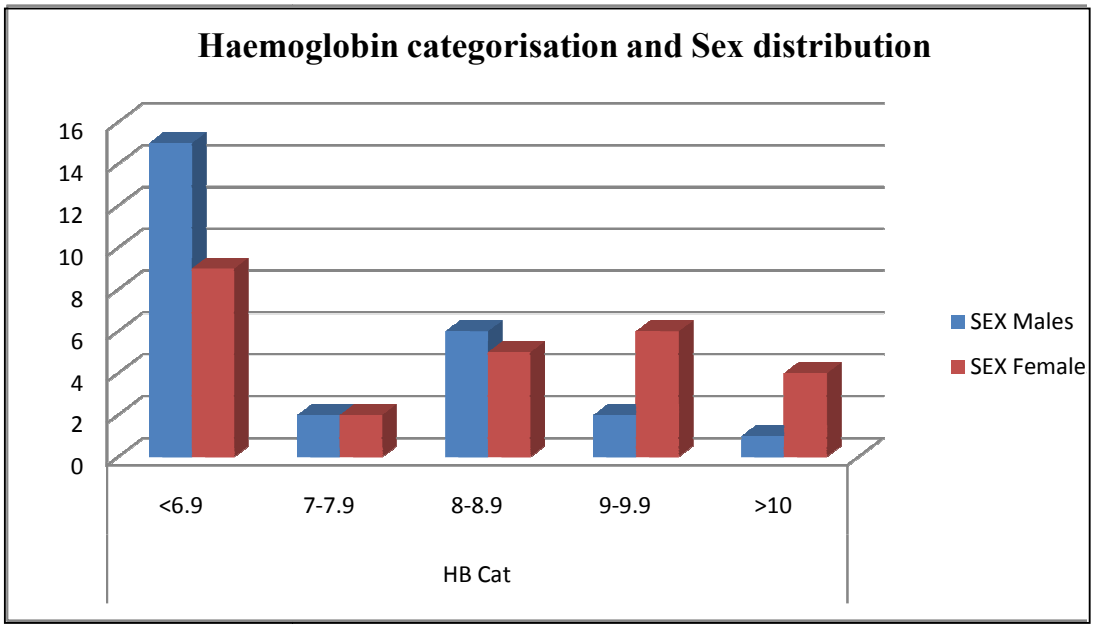


The base line CD4 among the study population was more than 200 cells in 44.2 % and between (50-199) it was 48.1 % and less than 50 cells were 7.7 % .The p value was 0.489 and there was no significant relationship between these 2 variables in this study.

6. Haemoglobin categorisation and Sex distribution

HB Categorisation	SEX		Total	
	Males	Females		Percent
<6.9	15	9	24	46.2
7-7.9	2	2	4	7.7
8-8.9	6	5	11	21.2
9-9.9	2	6	8	15.4
>10	1	4	5	9.6
Total	26	26	52	

P value - 0.249

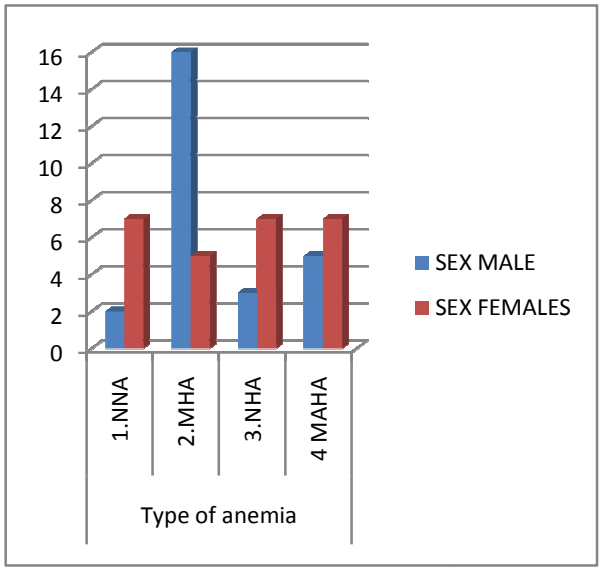


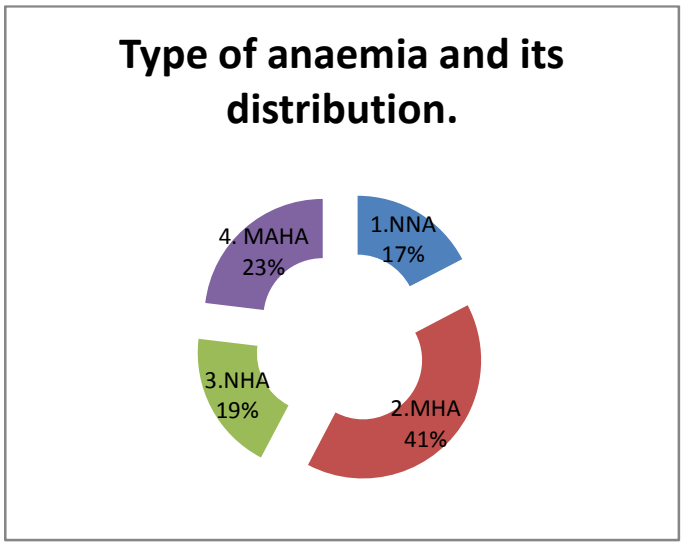
The patients with severe anaemia 46.2 % and the majority of them were males and there was no significant correlation between the anaemia and the sex of the patient.

5. **TYPE OF ANAEMIA AND SEX DISTRIBUTION.**

Type Of Anaemia	Sex		Total
	MALE	FEMALES	
1.Normocytic Normochromic An	2	7	9
2.Microcytic Hypochromic An	16	5	21
3. Normocytic Hypochrromic An	3	7	10
4 Macrocytic Hypochromic An	5	7	12
Total	26	26	52

P VALUE - **0.015**



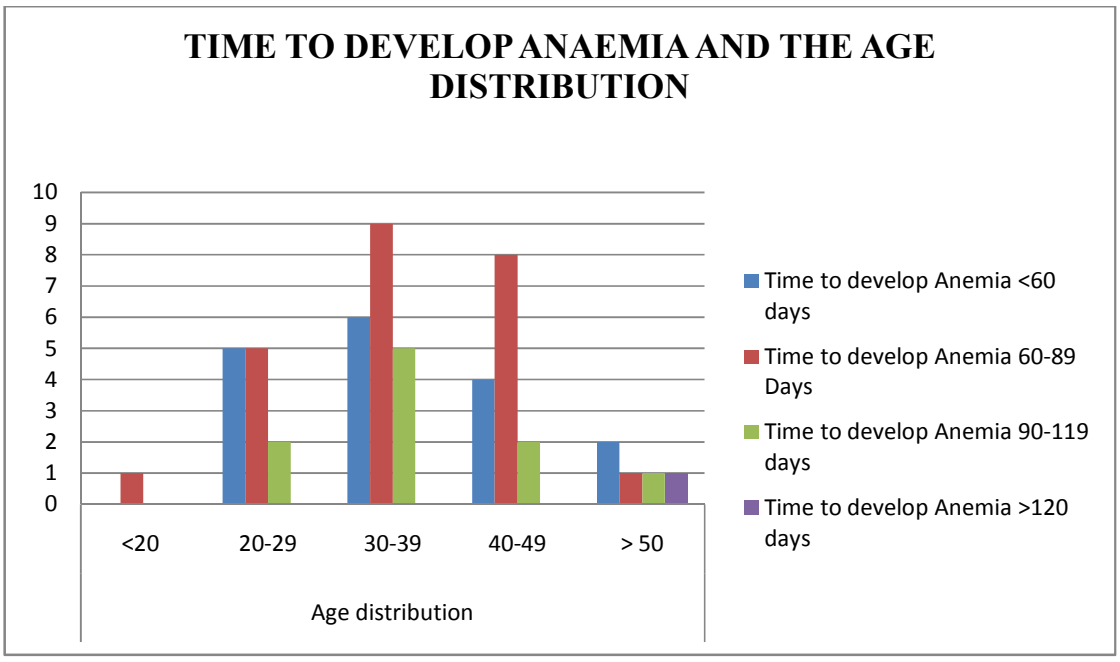


The most common type of Anaemia in the study was Microcytic Hypo chromic Anaemia found in 41% of population and when it was compared with sex there was a significant correlation (P Value of **0.015)** between the two variables. The second most common pattern was that of Macrocytic Hypo chromic Anaemia in about 23 % of patients. Overall the Hypo chromic pattern was predominantly found among the study population.

6. TIME TO DEVELOP ANAEMIA AND THE AGE DISTRIBUTION

Time to develop Anemia	Age distribution					Total	Percentages
	<20	20-29	30-39	40-49	> 50		
<60 days	0	5	6	4	2	17	32.7
60-89 Days	1	5	9	8	1	24	46.2
90-119 days	0	2	5	2	1	10	19.2
>120 days	0	0	0	0	1	1	1.9
Total	1	12	20	14	5	52	

P value - 0.386

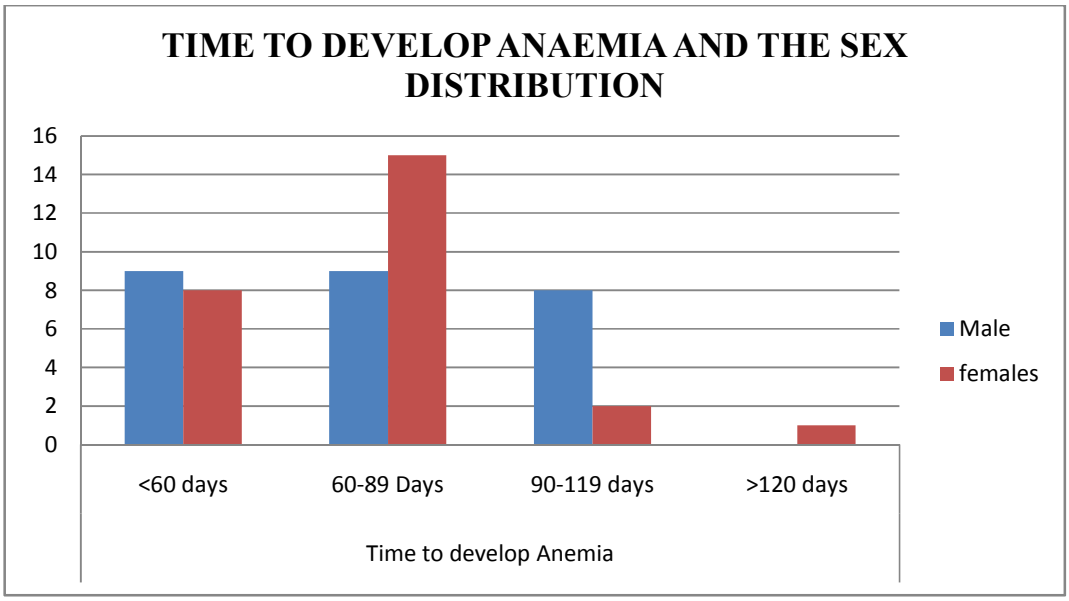


In The study 32.7 % developed anaemia in less than 60 days and 46.2 % of people developed anaemia in 60- 89 days ,it was > 90 days in 19.2 % of patients and there was no significance (p-.386) between the time to develop anaemia and the age categorisation.

7. TIME TO DEVELOP ANAEMIA AND THE SEX DISTRIBUTION

Time to develop Anemia	Sex		Total
	Male	females	
<60 days	9	8	17
60-89 Days	9	15	24
90-119 days	8	2	10
>120 days	0	1	1
Total	26	26	52

P value 0 .104

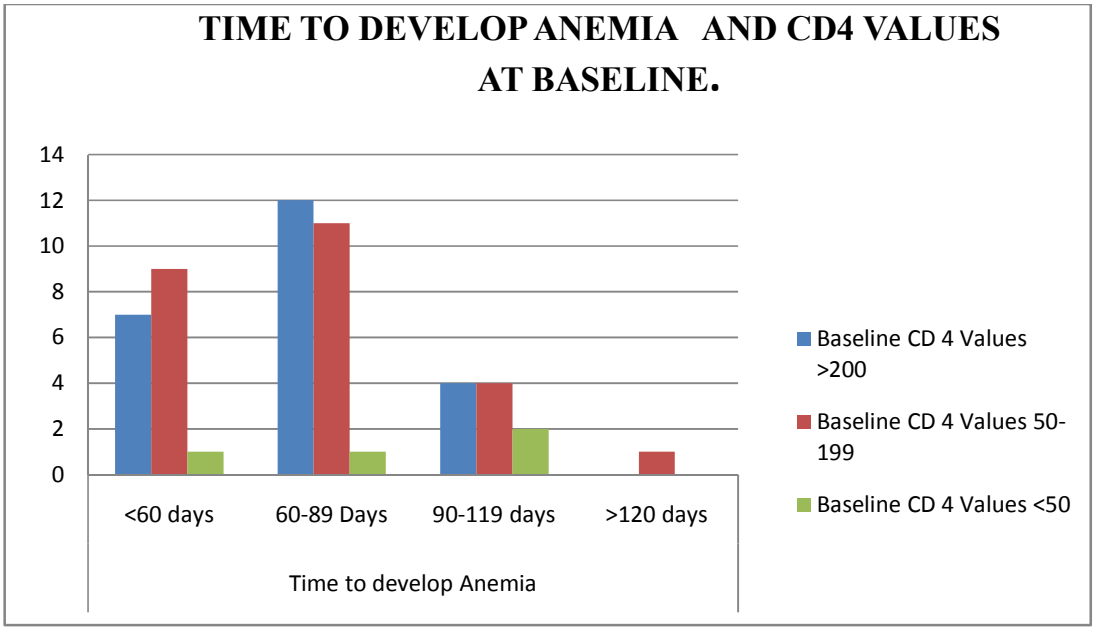


There was no statistical significance seen (p value of 0.104) between the time to develop anaemia and the sex distribution. It was observed that the females developed the anaemia more between 60-90 days but there was no statistical significance correlation.

8. TIME TO DEVELOP ANEMIA AND CD4 VALUES AT BASELINE.

Time to develop Anaemia	Baseline CD 4 Values			Total
	>200	50-199	<50	
<60 days	7	9	1	17
60-89 Days	12	11	1	24
90-119 days	4	4	2	10
>120 days	0	1	0	1
Total	23	25	4	52

P VALUE - 0.675

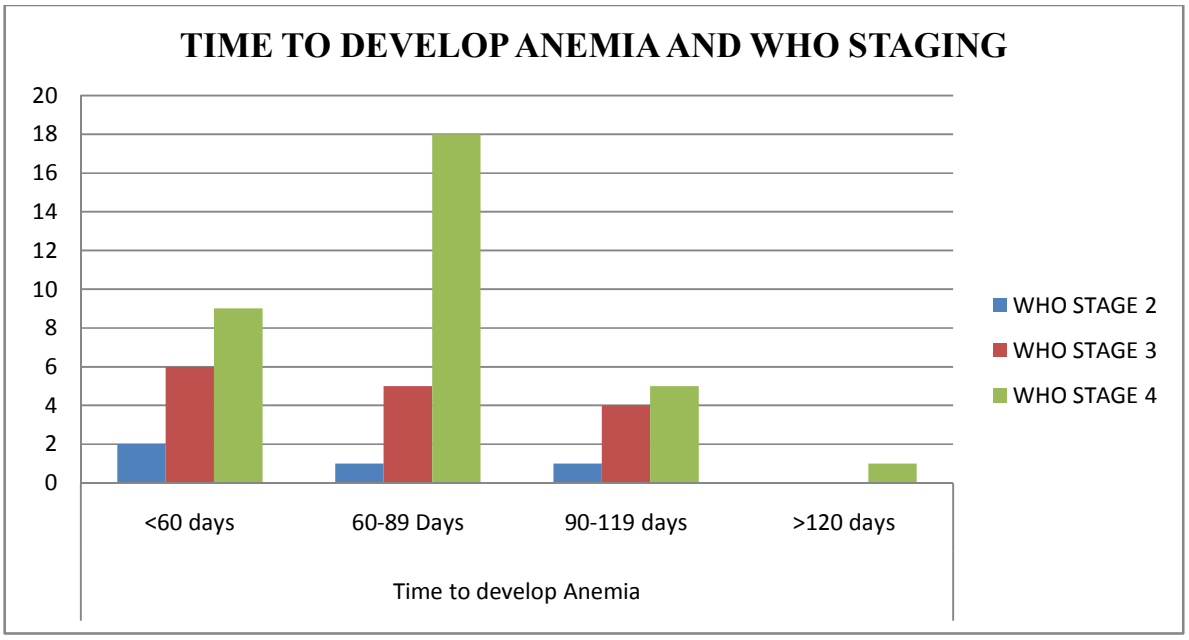


When Time to develop anaemia was compared with the CD4 counts at baseline it was observed that 78.8 % of the study patients developed anaemia in less than 90 days and there was no significant correlation between the two variables.

9. TIME TO DEVELOP ANEMIA AND WHO STAGING

Time to develop Anemia	WHO STAGE			Total
	2	3	4	
<60 days	2	6	9	17
60-89 Days	1	5	18	24
90-119 days	1	4	5	10
>120 days	0	0	1	1
Total	4	15	33	52

P.VALUE 0.718



When the WHO staging at base line was compared with time to develop anaemia it was observed that 34.6% of patients in the stage 4 category developed anaemia between 60-89 days and there was no significant correlation.

10. TIME TO DEVELOP ANEMIA AND THE TYPE OF ANEMIA

Time to develop Anemia	Type of anemia				Total
	NNA	MHA	NHA	MAHA	
<60 days	3	7	5	2	17
60-89 Days	6	7	3	8	24
90-119 days	0	7	1	2	10
>120 days	0	0	1	0	1
Total	9	21	10	12	52

P.VALUE 0.134

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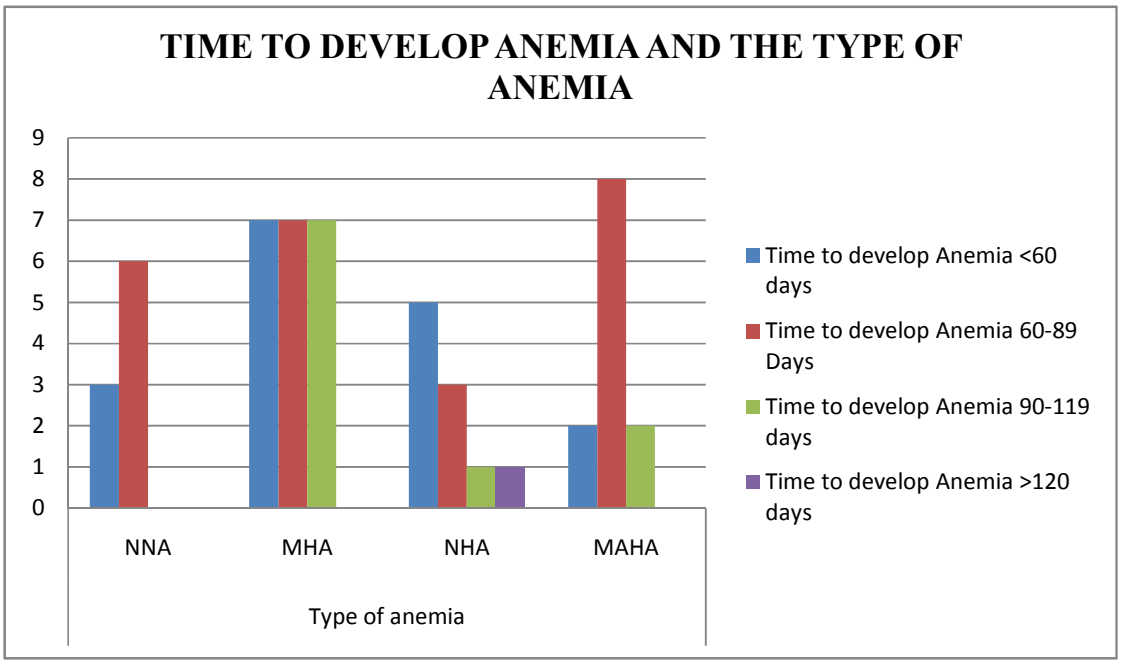
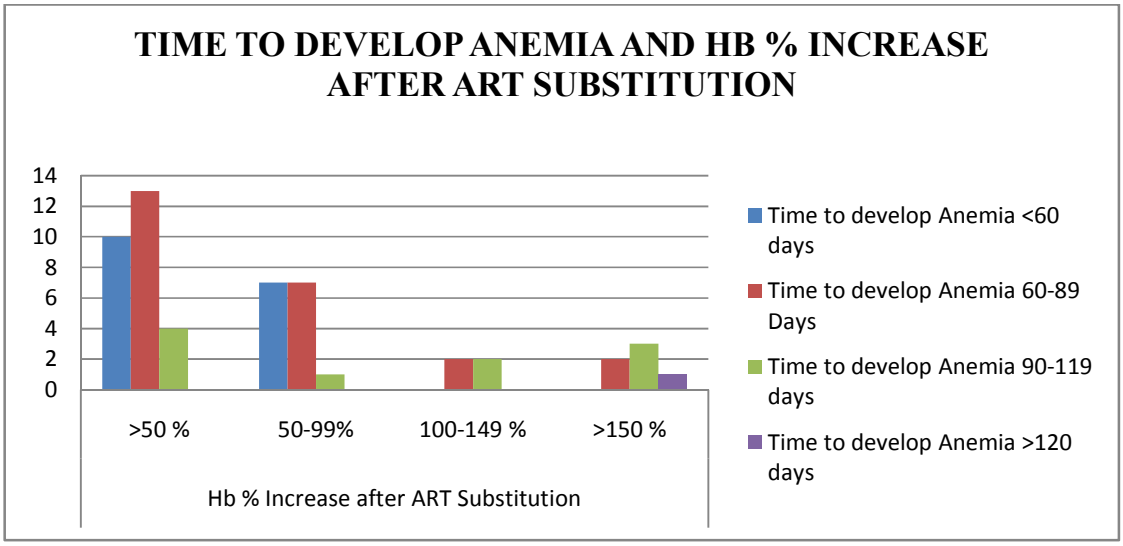


When the type of anaemia and time to develop anaemia was compared it was observed that distribution of Microcytic hypo chromic anaemia was equally distributed between <60 ,60-89 ,and 90-119 days.

11. TIME TO DEVELOP ANEMIA AND HB % INCREASE AFTI SUBSTITUTION

Time to develop Anemia	Hb % Increase after ART Substitution				Total
	>50 %	50-99%	100-149	>150	
<60 days	10	7	0	0	17
60-89 Days	13	7	2	2	24
90-119 days	4	1	2	3	10
>120 days	0	0	0	1	1
Total	27	15	4	6	52

P VALUE 0.028

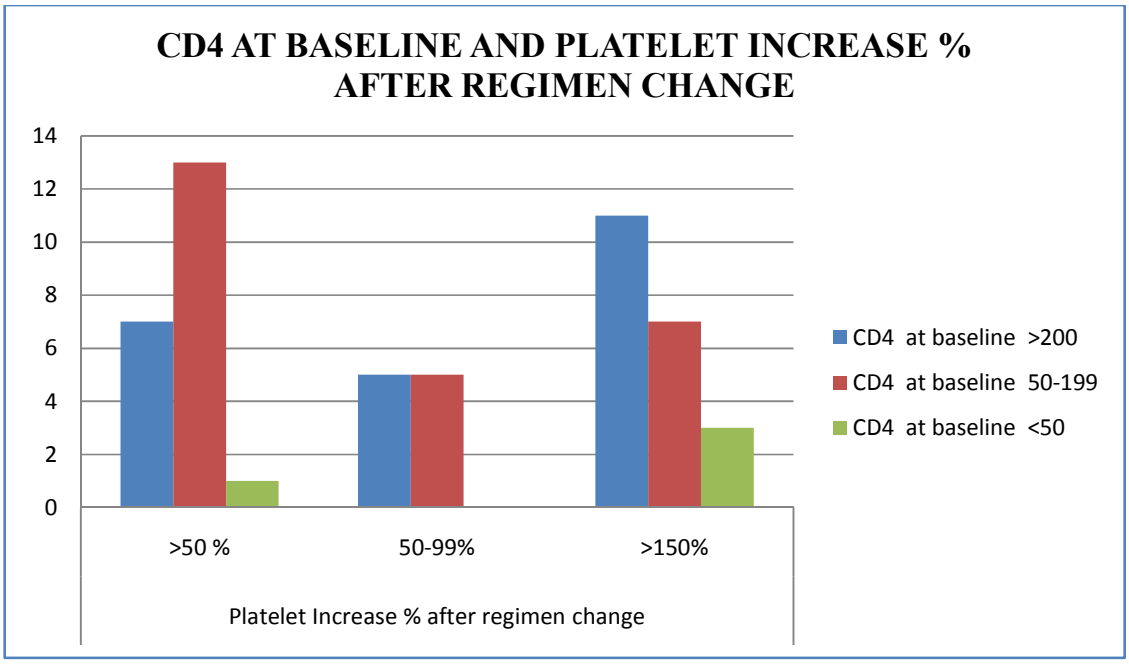


When the Hb rise after substitution was compared with the time to develop anaemia there was a (significant P value = 0.028) between the more than 50% rise and the time to development between 60-89 days.

12. CD4 AT BASELINE AND PLATELET INCREASE % AFTER REGIMEN CHANGE

CD4 at baseline	Platelet Increase % after regimen change			Total
	>50 %	50-99%	>150%	
>200	7	5	11	23
50-199	13	5	7	25
<50	1	0	3	4
Total	21	10	21	52

P VALUE 0.294

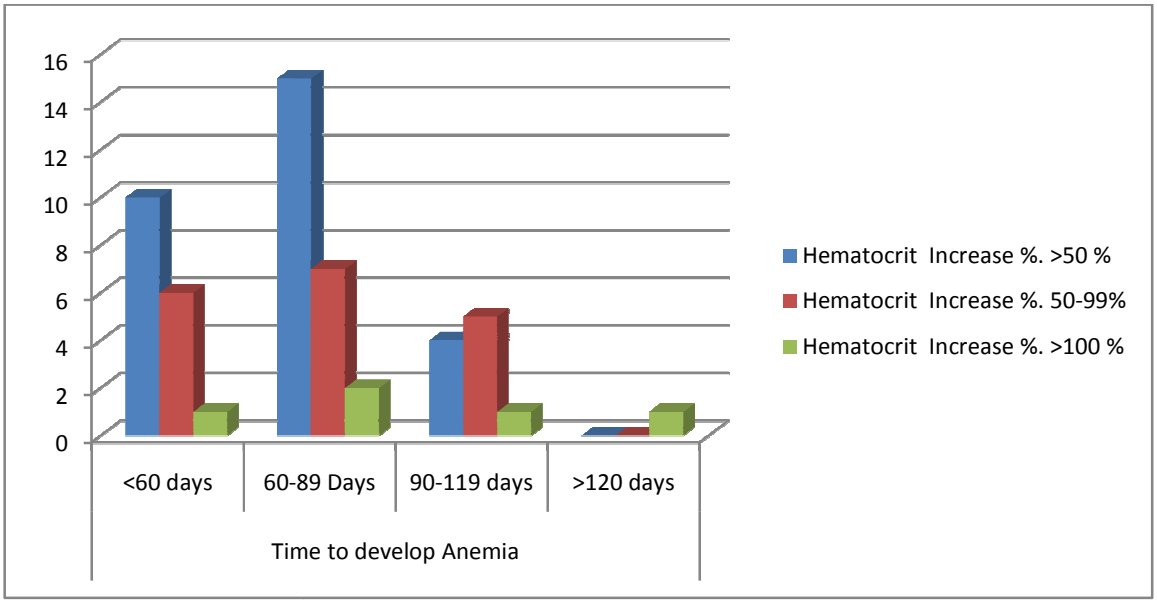


When the platelet increase % after the regimen change was correlated with the CD4 at baseline there was no significant correlation observed (P value of =0.294) between the comparing variables.

13. TIME TO DEVELOP ANEMIA AND HEMATOCRIT INCREASE

Time to develop Anemia	Hematocrit Increase %.			Total
	>50 %	50-99%	>100 %	
<60 days	10	6	1	17
60-89 Days	15	7	2	24
90-119 days	4	5	1	10
>120 days	0	0	1	1
Total	29	18	5	52

P VALUE **0.081**

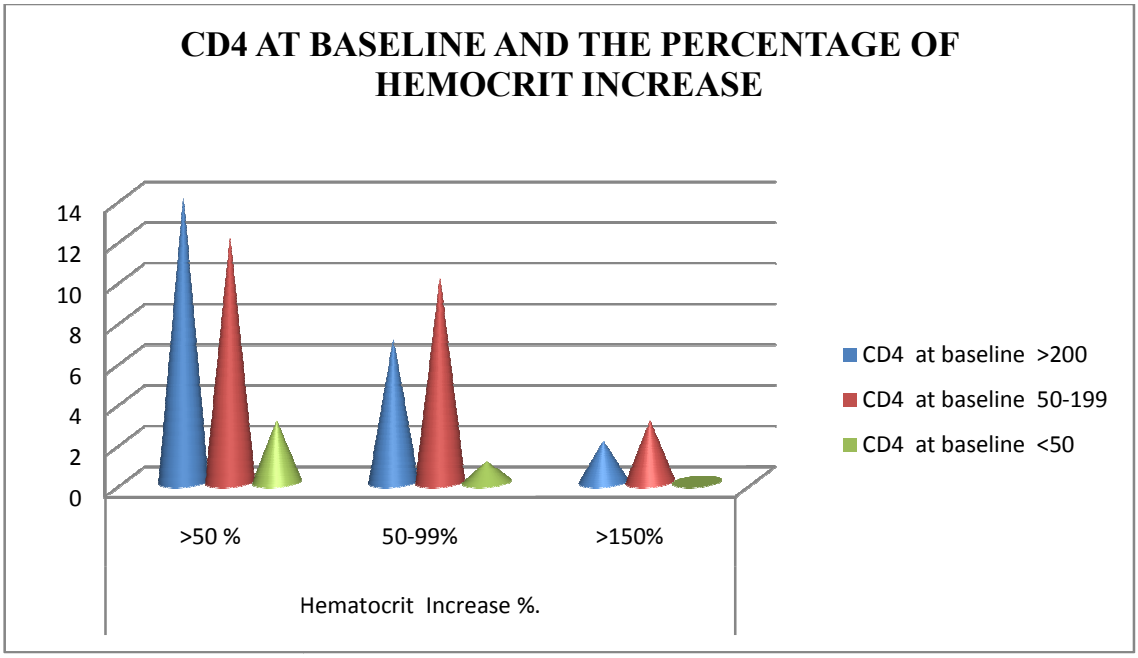


When the Hematocrit rise was compared with the time to develop anaemia it was observed that there was a significant rise (p value =0.081) in the 60-89 days group .

14. CD4 AT BASELINE AND THE PERCENTAGE OF HEMOCRIT INCREASE

CD4 at baseline	Hematocrit Increase %. >50 %	50-99%	>150%	Total
>200	14	7	2	23
50-199	12	10	3	25
<50	3	1	0	4
Total	29	18	5	52

P VALUE = 0.804

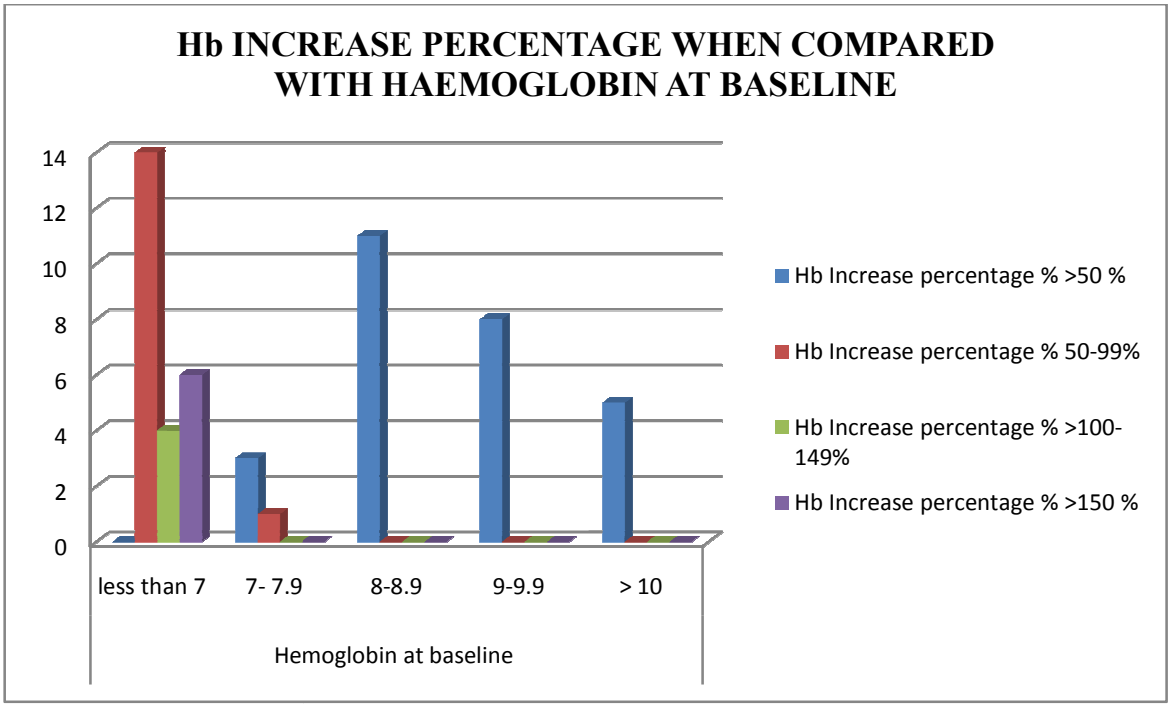


When the percentage increase of Hematocrit was compared with the base line CD4 counts there was no significant correlation between the comparing variables.

15. Hb INCREASE PERCENTAGE WHEN COMPARED WITH HAEMOGLOBIN AT BASELINE

Hb Increase percentage	Hemoglobin at baseline					Total
	< 7	7- 7.9	8-8.9	9-9.9	>10	
>50 %	0	3	11	8	5	27
50-99%	14	1	0	0	0	15
>100- 149%	4	0	0	0	0	4
>150 %	6	0	0	0	0	6
Total	24	4	11	8	5	52

P VALUE 0.000

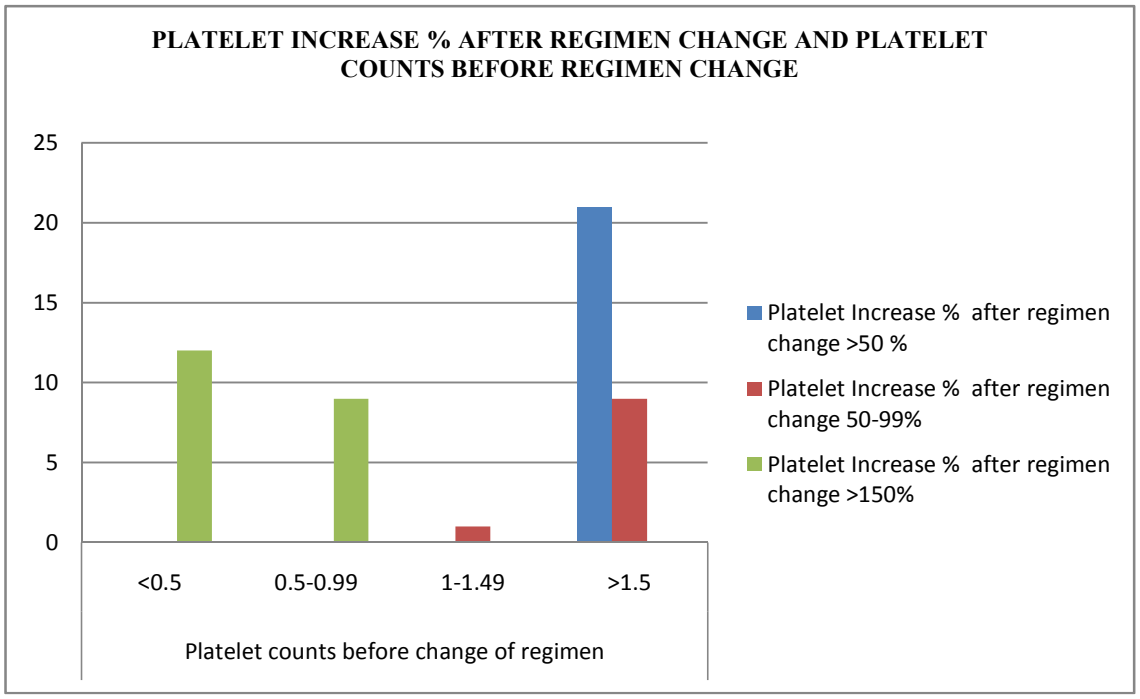


When the Haemoglobin percentage increase was compared with base line Haemoglobin there was a significant correlation (P=0.000) between the percentage rise of 50-99 % and the baseline Hb of less than 7.

16. PLATELET INCREASE % AFTER REGIMEN CHANGE AND PLATELET COUNTS BEFORE REGIMEN CHANGE

Platelet Increase % after regimen change	Platelet counts before change of regimen				Total
	<0.5	0.5-0.99	1-1.49	>1.5	
>50 %	0	0	0	21	21
50-99%	0	0	1	9	10
>150%	12	9	0	0	21
Total	12	9	1	30	52

P VALUE **0.000**



When the platelet increase percentage from the base line was compared with its base line value following the substitution of the ART it was observed that there was a significant correlation (p= 0.000) of more than 50% increase and the baseline value of >1.5 lacs.

Peripheral Smear Findings

The Peripheral study mirrored with the findings found in the cell counts with Hypo chromic anaemia the most common findings in 83 % (43) of patients and normocytic anaemia in 17 % of the patients. Macrocytosis were found among 23 % of patients. Platelets were reduced in numbers in 12 (23 %) of patients and Pancytopenia in 7 patients (11%) who were subjected to bone marrow aspiration.

Bone marrow findings

Bone marrow aspiration for done in 7 of the 52 patients and the findings were similar in all of them. There was hyper cellular bone marrow with a increased myeloid Erythroid ratio in 6 of the patients. There was no evidence of myelodysplastic changes and abnormal cells in the marrow. Bone marrow culture for did not reveal any opportunistic infections. In one of the patient there was a vacuolization of the neutrophil precursors with decrease in cells of the Erythroid lineage. In other patients Bone marrow aspiration was found unnecessary as there were no indications and in 22 patients did not consent for the invasive study.

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DISCUSSION

52 patients presented with anaemia with equal distribution among both sexes and all of them were found to be initiated on the AZT based regimen. This is in common with other studies which had shown AZT has the primary agent causing anaemia.

The age in the study group ranged from 18-56 with 85 % in the reproductive age group which is similar to various other studies which had demonstrated the occurrence of the epidemic in the reproductive age group and there was no statistical significance noted with age and categorisation and the incidence of haematological changes in this study.

Besides Anaemia there were 13% incidence of Pancytopenia which is considerably low when compared with studies done by Ellaurie et al and Castella et al in which the incidence was surprisingly higher at around 50 %.There was no significant correlation between the occurrence of Pancytopenia and the baseline CD4 counts which was observed in other studies.

The incidence of Thrombocytopenia was 42 % which was similar to the studies conducted by Zon Li et al and Murphy Et al in which there was a 40% incidence was noted. It was also noted in the Multicenter AIDS cohort study the incidence of thrombocytopenia ranged from 13 - 61 %. There was a significant increase in the rise from the base line of the

platelet count when it was compared with its base line value following the substitution of the ART and it was observed that there was a significant correlation (p= 0.000) of more than 50% increase and the baseline value of >1.5 lacs which is unique to this study.

The platelet count normalised within a period of 6 months after substitution of the AZT based regimen with Tenofovir based regimen. Of the patients who developed severe thrombocytopenia all were found to have CD4 counts distributed equally below and above 200.

Signs and Symptoms.

Among the clinical symptoms Fatigue and dyspnoea were found in 34 % of patients and this was on par with other studies and among the patients who had fatigue and Dyspnoea ,the incidence of palpitation was 61%.Diarrrohea was seen in 17 % of patients and Jaundice was not found in any one.

There were 19 % of patients who had a high BP >140/90 and all of them were found to be above 40 age group and were on antihypertensive when the patient were started on ART.

The incidence of anaemia and the type of Anaemia .

All of the patients were on AZT before they developed anaemia and they were all transferred to a Tenofovir based regimen and they showed improvement in the haematological parameters. The percentage of severe anaemia <7 were 46.2 % in the study population and which was in accordance with the studies conducted by Aboulafia et al (70%) and when compared with the those with having CD4 <50 it was found in only 7% of the study population, which meant that the study population had been started on ART before they reached a severe immunological depletion of CD4 counts.

The most common type of Anaemia in the study was Microcytic Hypo chromic Anaemia found in 41% of population and when it was compared with sex there was a significant correlation (P Value of **0.015) betw**een the two variables. The second most common pattern was that of Macrocytic Hypo chromic Anaemia in about 23 % of patients. Overall the Hypo chromic pattern was predominantly found among the study population.

Another interesting observation that was made was the significant rise in haemoglobin and Hematocrit after the initial drop due to AZT and it was observed especially in those patients who developed anaemia

within 90 days of ART. This significant rise in Hb and Hematocrit happened over a period of 6 months.

The bone marrow findings did not bring about any significant correlation and they were on par with other studies .There was hyperrcellularity with increased myeloid Erythroid ration in 85 % of the patients who underwent bone marrow aspiration .

CONCLUSION

1. There is a significant rise in Haemoglobin and the Hematocrit within a median time duration of 6 months after the change from AZT based regimen.

2. The Baseline CD4 counts, Age and Sex , did not significantly contribute to the development of Cytopenias in this study but the AZT based regimen had a significant impact contributing to all the cases in the study population.

SUMMARY

1. The age in the study group ranged from 18-56 with 85 % in the reproductive age group which is similar to various other studies.

2. The percentage of severe anaemia <7 were 46.2 % in the study population and which was in accordance with other studies.

3. The most common type of Anaemia in the study was Microcytic Hypo chromic Anaemia found in 41% of population.

4. Besides Anaemia there were 13% incidence of Pancytopenia which is considerably low and the incidence of Thrombocytopenia was 42 % which was similar to other studies.

5. There was no significant correlation between the occurrence of Pancytopenia and the baseline CD4 counts which was observed in other studies

6. The second most common pattern was that of Macrocytic Hypo chromic Anaemia in about 23 % of patients.

7. Among the clinical symptoms Fatigue and dyspnoea were found in 34 % of patients and this was on par with other studies.

8. There was a significant rise in haemoglobin and Hematocrit after the initial drop due to AZT and it was observed especially in those patients who developed anaemia within 90 days of ART.

9. The platelet count normalised within a period of 6 months after substitution of the AZT based regimen with Tenofovir based regimen.

10. The bone marrow findings did not bring about any significant correlation and they were on par with other studies.

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PROFORMA

Name:

Age:

Sex:

Occupation:

Address:

Presenting illness:

H/O Anaemia following HAART initiation.

Personal history:

H/O Alcohol intake

Duration

Type

How often

Units of alcohol/day

H/O Drug Intake (ATT ,previous ART,2 and line HAART,

Past history:

H/O Myelosuppressive drugs (other than Cotrimoxazole)

IRIS after initiation of HAART.

Haematological problems (Malignancies, Hereditary disorders, anaemia of Chronic Disease)

Treatment for both HIV and TB.

HIV and CMV.

HIV and Hep B and Hep C infections.

H/O Second Line HAART ,Patients with Renal Failure

H/O HIV -2 infections

H/O poor adherence (< 80%).

Family history:

H/O Systemic hypertension, Diabetes mellitus.

H/O Hereditary disorders in the family

GENERAL EXAMINATION:

Built:

Height:

Weight:

Pallor, Icterus, Clubbing, Cyanosis

Lymphadenopathy, Pedal edema.

Vital signs PR: RR:

BP: Temp:

SYSTEMIC EXAMINATION:

Cardio vascular system

Respiratory system

Central nervous system

Investigations.

Complete Blood counts (Automated).

CD 4 counts .

Peripheral smear

Bone marrow examination.

KEY TO MASTER CHART

Symptoms

0 – No Symptoms

1 – Yes Symptoms

MCH Category

0 to 25 = 1

25 – 30 = 2

- 30 = 3

Sex

1 = Male

2 = Female

Anaemia

1 = Presence of Anaemia

0 = Absence of Anaemia

MCV

< 80 = Microcytic

80-100 = Normocytic

- 100 = Macrocytic

ZLN

Zidovudine / Lamivudine / Nevirapine

BP < 140/80 = 0 = Normal

> 140/80 =1 = High BP

ஒப்புதல் படிவம்

பெயர் :

பாலினம் :

முகவரி : வயது :

அரசு கோவை மருத்துவக் கல்லூரியில் பொது மருத்துவ துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவர் **மரு. பு,க.இ.ராமலிங்கம்** அவர்கள் மேற்கொள்ளும் "**கோயமுத்தூர் மருத்துவ கல்லூரி மருத்துவமனையில் எச் ஐ வி நோயினால் பாதிக்கப்பட்ட நோயாளிகளுக்கு முதல் நிலை ART மருந்து கொடுப்பதினால் இரத்தத்தில் உள்ள அணுக்களில் ஏற்படும் மாறுதல் பற்றிய** " ஆய்வில் செய்முறை மற்றும் அனைத்து விவரங்களையும் கேட்டுக் கொண்டு எனது சந்தேகங்களை தெளிவுபடுத்திக் கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன்.

நான் இந்த ஆய்வில் முழு சம்மதத்துடன், சுய சிந்தனையுடனும் கலந்து கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் என்னுடைய அனைத்து விபரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதை தெரிவித்துக்கொள்கிறேன். எந்த நேரத்தில் அந்த ஆய்விலிருந்து நான் விலகிக் கொள்ள எனக்கு உரிமை உண்டு என்பதையும் அறிவேன்.

இடம் : கையொப்பம் / ரேகை

நாள் :

INFORMED CONSENT

DEPARTMENT OF GENERAL MEDICINE

Coimbatore Medical College, Coimbatore

Principal investigator : Dr. Ramalingam. P.K.

Research guide : Dr. Kumar Natarajan. M.D

Organization : Department of General Medicine

Informed consent : I have been invited to participate in research Project titled **'A STUDY ON HAEMATOLOGICAL CHANGES FOLLOWING FIRST LINE HAART"** I understand, it will be answering a set of questionnaire, undergo physical examination, investigations and appropriate treatment.

I also give consent to utilize my personal details for study purpose and can be contacted if necessary.

I am aware that I have the right to withdraw at any time which will not affect my medical care.

Name of the participant:

Signature:

Date

ART ID	NAME	AGE	Age Cat	SEX	CD4 Int	CD4 Int Cat	CD4 OCUN	WHO STAG	FEVER (0=I	WEIGHT LC	ANEMIA (C	JAUNDICE (	FATIGUE ((
A2894	Lalitha	55	5	2	134	2	256	3	0	0	1	0	1
A2933	K.Prakash	40	4	1	273	1		2	1	1	1	0	1
A3137	Rabin Raj	33	3	1	232	1		3	0	0	1	0	1
A3186	Siddhik	33	3	1	230	1		4	1	1	1	0	1
A3225	Venkadapathy	55	5	1	188	2		4	0	0	1	0	1
A3329	Nagarani .R	36	3	1	219	1		4	0	0	1	0	1
A3424	Ranjith kumar	31	3	1	253	1		4	0	0	1	0	1
A3439	Latha.B	35	3	2	89	2	234	4	0	0	1	0	1
A3527	Gunasekaran.M	34	3	1	696	1		4	0	0	1	0	1
A3547	Muralidharan.N	47	4	1	182	2		4	0	0	1	0	1
A3590	Devi	27	2	2	175	2	345	4	0	0	1	0	1
A3866	Duraisamy	43	4	1	215	1		3	0	0	1	0	1
A3906	Selvaraj	34	3	1	198	2	247	4	0	0	1	0	1
A4044	Arumugam,R	38	3	1	299	1		3	0	0	1	0	1
A4069	Suresh	30	3	1	196	2		3	0	0	1	0	1
A4108	Aruchasamy	41	4	1	165	2		4	1	1	1	0	1
A4120	Chitra.A	29	2	2	196	2		4	0	0	1	0	1
A4144	Shanmugam	56	5	1	94	2	198	3	0	0	1	0	1
A4148	Krishnakumar.k .r	44	4	1	202	1	456	2	1	1	1	0	1
A4152	Ashokan	45	4	1	40	3	246	4	0	0	1	0	1
A4153	Sheeba	38	3	2	194	2	289	4	1	1	1	0	1
A4163	Jegathambal	28	2	2	109	2	460	4	0	0	1	0	1
A4196	Ponnusamy. N	43	4	1	186	2		4	0	0	1	0	1
A4200	Thangamani	55	5	2	76	2	245	4	0	0	1	0	1
A4278	P.Selvi	25	2	1	244	1		2	0	0	1	0	1
A4322	Soundaraj.M	51	5	1	194	2		3	0	0	1	0	1
A4336	Ramathal.S	43	4	2	103	2	345	4	0	0	1	0	1
A4340	Jaithal.K	13	1	2	51	2	134	4	0	0	1	0	1
A4354	Sudha	29	2	2	65	2	265	4	1	1	1	0	1
A4396	Saraswathy	30	3	2	68	2	278	4	1	1	1	0	1
A4402	Mary	40	4	2	340	1	408	2	1	1	1	0	0
A4405	Saraswathi	30	3	2	243	1	340	4	1	1	1	0	0
A4407	Parameswari.K	28	2	2	272	1		4	1	1	1	0	1
A4447	Indra.R	42	4	2	291	1	403	4	1	1	1	0	0
A4450	Indhirani. P	29	2	2	314	1	345	4	1	1	1	0	0
A4472	Krishnaveni.M	30	3	2	292	1		4	1	1	1	0	1
A4477	Geetha.R	28	2	2	301	1		3	1	1	1	0	1
A4539	Gomathy.K	37	3	2	63	2	200	3	0	0	1	1	0
A4585	Raj. D	48	4	1	276	1		3	1	1	1	0	0
A4591	Vimalraj.P	32	3	1	192	2		4	0	0	1	0	0
A4638	Radha	34	3	2	48	3	202	3	1	1	1	0	0
A4648	Prithivi.M	49	4	1	329	1		4	0	0	1	1	0
A4651	Vijayalakshmi. R	35	3	2	124	2	246	3	1	1	1	0	1
A4671	Chithra.C	24	2	2	43	3	290	4	0	0	1	0	0
A4675	Balasubramani am	40	4	1	167	2		3	1	1	1	0	1
A4704	Radhika.A	40	4	2	328	1		4	0	0	1	0	0
A4726	Kousalya.N	35	3	2	310	1		4	1	1	1	0	1
A4829	Sathishkumar.C	34	3	1	115	2	240	3	0	0	1	0	0
A4849	Chinnasamy. A	22	2	1	233	1		4	1	1	1	0	1
A4868	Kadeswari	25	2	2	295	1		3	0	0	1	0	1
A4872	Rajendran.P	37	3	1	135	2		4	0	0	1	0	1
A5148	BEENA.J	24	2	2	39	3	309	4	0	0	1	0	0

0	1
20	2
30	3
40	4
50	5

0	3
50	2
200	1

ART ID	NAME	DYSPNEA (	ANOREXIA	COUGH (0	DIARRHOE	PALPITATIC	Pulse rate	BP(0= NOF	TEMPERAT	PALLOR (0	CYANOSIS (
A2894	Lalitha	1	1	0	0	0	90	1	0	1	0
A2933	K.Prakash	1	1	0	0	1	98	0	0	1	0
A3137	Rabin Raj	1	1	0	0	0	96	0	0	1	0
A3186	Siddhik	1	0	0	0	1	96	0	0	1	0
A3225	Venkadapathy	1	0	1	0	1	88	1	0	1	0
A3329	Nagarani .R	0	1	0	0	0	75	0	0	1	0
A3424	Ranjith kumar	0	0	0	0	0	88		0	1	0
A3439	Latha.B	0	1	0	0	0	80	0	0	1	0
A3527	Gunasekaran.M	0	0	0	0	1	110	0	0	1	0
A3547	Muralidharan.N	1	1	0	0	0	74	1	0	1	0
A3590	Devi	1	1	0	0	0	88	0	0	1	0
A3866	Duraisamy	0	0	0	1	0	78			1	0
A3906	Selvaraj	1	0	1	0	0	94	0	0	1	0
A4044	Arumugam,R	0	1	0	1	1	100	0		1	0
A4069	Suresh	0	1	0	1	0	70	1		1	0
A4108	Aruchasamy	1	0	0	0	1	88	0	0	1	0
A4120	Chitra.A	0	0	1	1	0	80	0		1	0
A4144	Shanmugam	1	0	0	0	0	94	1	0	1	0
A4148	Krishnakumar.k .r	1	1	1	1	1	104	1	0	1	0
A4152	Ashokan	1	0	0	0	1	90	1	0	1	0
A4153	Sheeba	0	0	1	0	0	70	0	0	0	0
A4163	Jegathambal	0	1	0	0	0	72	0	0	0	0
A4196	Ponnusamy. N	1	0	0	0	1	108	0	0	0	0
A4200	Thangamani	1	1	0	0	1	98	0	0	0	0
A4278	P.Selvi	0	0	1	0	0	78	0	0	0	0
A4322	Soundaraj.M	0	0	1	0	0	89	1	0	1	0
A4336	Ramathal.S	1	1	0	0	1	100	0	0	0	0
A4340	Jaithal.K	0	0	0	0	0	74	0	0	0	0
A4354	Sudha	0	0	1	1	0	76	0	0	0	0
A4396	Saraswathy	1	1	0	1	0	80	0	0	0	0
A4402	Mary	0	0	0	0	0	88	0	0	0	0
A4405	Saraswathi	0	0	0	0	0	80	0	0	0	0
A4407	Parameswari.K	1	1	0	0	0	90	0	0	0	0
A4447	Indra.R	0	0	0	0	0	92	1	0	0	0
A4450	Indhirani. P	0	1	0	0	0	68	0	0	0	0
A4472	Krishnaveni.M	0	0	1	0	0	78	0	0	0	0
A4477	Geetha.R	0	0	0	0	0	64	0	0	0	0
A4539	Gomathy.K	0	0	0	0	0	90	0	0	0	0
A4585	Raj. D	0	1	0	0	0	78	0	0	0	0
A4591	Vimalraj.P	0	0	0	0	0	76	0	0	0	0
A4638	Radha	0	1	0	0	0	86	0	0	0	0
A4648	Prithivi.M	0	0	0	1	0	88	0	0	0	0
A4651	Vijayalakshmi. R	0	1	1	0	0	88	0	0	0	0
A4671	Chithra.C	0	0	0	0	0	70	0	0	0	0
A4675	Balasubramani am	0	0	0	0	0	76		0	0	0
A4704	Radhika.A	0	0	0	0	0	74	0	0	0	0
A4726	Kousalya.N	1	1	0	0	0	68	0	0	0	0
A4829	Sathishkumar.C	0	0	0	0	0	90	1	0	0	0
A4849	Chinnasamy. A	0	1	1	0	0	78		0	0	0
A4868	Kadeswari	0	0	0	0	0	90		0	0	0
A4872	Rajendran.P	0	1	1	1	0	88		0	0	0
A5148	BEENA.J	0	0	0	0	0	80	0	0	0	0

ART ID	NAME	EMACIATION(	EDEMA (0= NO	O THRUSH (0=I	PETECHEIAE(0	CVS (0=NO SYI	RS (0=NO SYMI	PA (0=NO SYM	CNS (0=NO SYI	HB	HB Cat	
A2894	Lalitha	1	1	0	0	0	0	0	0	6	1	
A2933	K.Prakash	1	1	1	1	0	0	0	0	5	1	
A3137	Rabin Raj	0	1	1	0	0	0	0	0	8	3	
A3186	Siddhik	0	0	0	0	0	0	0	0	6	1	
A3225	Venkadapathy	1	0	1	0	0	0	0	0	8	3	
A3329	Nagarani .R	0	0	0	0	0	0	0	0	5	1	
A3424	Ranjith kumar	0	0	0	0	0	0	0	0	7	2	
A3439	Latha.B	0	0	0	0	0	0	0	0	8	3	
A3527	Gunasekaran.M	0	1	0	0	0	0	0	0	4	1	
A3547	Muralidharan.N	0	0	0	0	0	0	0	0	8	3	
A3590	Devi	1	0	0	1	0	0	0	0	6	1	
A3866	Duraisamy	0	0	0	0					7	2	
A3906	Selvaraj	1	1	1	0	0	0	0	0	6	1	
A4044	Arumugam,R	0	1	0	0					3	1	
A4069	Suresh	0	0	0	0					5	1	
A4108	Aruchasamy	0	1	0	0	0	0	0	0	4	1	
A4120	Chitra.A	1		0						6	1	
A4144	Shanmugam	1	1	1	1	0	0	0	0	4	1	
A4148	Krishnakumar.k.r	0	1	0	0					4	1	
A4152	Ashokan	1	0	0	0	0	0	0	0	5	1	
A4153	Sheeba	0	0		0	0	0	0	0	6	1	
A4163	Jegathambal	0	0	0	0	0	0	0	0	8	3	
A4196	Ponnusamy. N	0	1	0	0	0	0	0	0	2	1	
A4200	Thangamani	1	1	0	0	0	0	0	0	4	1	
A4278	P.Selvi	0	0	0	0	0	0	0	0	6	1	
A4322	Soundaraj.M	1	0	1	0	0	0	0	0	6	1	
A4336	Ramathal.S	0	1	0	0					4	1	
A4340	Jaithal.K	0	0	0	0	0	0	0	0	5	1	
A4354	Sudha	1	0	1	0					7	2	
A4396	Saraswathy	0	1	0	0	0	0	0	0	10.1	5	
A4402	Mary	0	0	0	1					10	5	
A4405	Saraswathi	0	0	0	0	0	0	0	0	9.1	4	
A4407	Parameswari.K	0	1	0	0	0	0	0	0	9.3	4	
A4447	Indra.R	0	0	0	0	0	0	0	0	9.4	4	
A4450	Indhirani. P	0	0	0	1	0	0	0	0	10.3	5	
A4472	Krishnaveni.M	1	0	0	0	0	0	0	0	8	3	
A4477	Geetha.R	0	0	1	0	0	0	0	0	6	1	
A4539	Gomathy.K	0	0	0	1	0	0	0	0	8.5	3	
A4585	Raj. D	0	0	0	0					10.4	5	
A4591	Vimalraj.P	0	0	0	0	0	0	0	0	9	4	
A4638	Radha	0	0	0	0					9.4	4	
A4648	Prithivi.M	0	0	0	0					9.3	4	
A4651	Vijayalakshmi. R	0	0	0	0	0	0	0	0	7	2	
A4671	Chithra.C	0	0	0	0					9.8	4	
A4675	Balasubramaniam	0	0	1	0	0	0	0	0	5	1	
A4704	Radhika.A	0	0	0	1					8.9	3	
A4726	Kousalya.N	0	1	0	1	0	0	0	0	9.8	4	
A4829	Sathishkumar.C	0	0	0	0	0	0	0	0	8.6	3	
A4849	Chinnasamy. A	0	0	0	0	0	0	0	0	8	3	
A4868	Kadeswari	0	0	1	0	0	0	0	0	6	1	
A4872	Rajendran.P	1	0	0	0	0	0	0	0	8	3	
A5148	BEENA.J	0	0	0	0	1	0	0	0	10.1	5	

0	1
7	2
8	3
9	4
10	5

ART ID	NAME	HEMATOCRIT	Hematocrit Cat	TC	TC cat	NEUTROPIL CO	Abs Neut	Abs Neut Cat	LYMPHOCYTE C	Abs Lymph	Abs Lymph
A2894	Lalitha	22	2	1500	2	60	900	2	16	240	
A2933	K.Prakash	18	1	800	1	54	432	1	18	144	
A3137	Rabin Raj	14	1	1200	2	70	840	2	21	252	
A3186	Siddhik	23	2	6800	4	72	4896	4	17	1156	
A3225	Venkadapathy	24	2	9200	4	80	7360	4	12	1104	
A3329	Nagarani .R	16	1	5800	4	82	4756	4	19	1102	
A3424	Ranjith kumar	18	1	11900	4	82	9758	4	14	1666	
A3439	Latha.B	26	2	10900	4	79	8611	4	20	2180	
A3527	Gunasekaran.M	22	2	9800	4	80	7840	4	12	1176	
A3547	Muralidharan.N	18	1	10900	4	90	9810	4	8	872	
A3590	Devi	21	2	1300	2	61	793	2	21	273	
A3866	Duraisamy	17	1	6700	4	55	3685	4	19	1273	
A3906	Selvaraj	20	2	12000	4	82	9840	4	19	2280	
A4044	Arumugam,R	14	1	9900	4	82	8118	4	14	1386	
A4069	Suresh	16	1	9800	4	80	7840	4	7	686	
A4108	Aruchasamy	16	1	11000	4	80	8800	4	12	1320	
A4120	Chitra.A	19	1	6800	4	90	6120	4	8	544	
A4144	Shanmugam	16	1	1700	2	61	1037	3	21	357	
A4148	Krishnakumar.k.r	16	1	8800	4	85	7480	4	9	792	
A4152	Ashokan	20	2	9800	4	84	8232	4	12	1176	
A4153	Sheeba	18	1	11000	4	72	7920	4	17	1870	
A4163	Jegathambal	24	2	6800	4	80	5440	4	12	816	
A4196	Ponnusamy. N	16	1	9200	4	82	7544	4	19	1748	
A4200	Thangamani	16	1	9900	4	80	7920	4	12	1188	
A4278	P.Selvi	16	1	11000	4	90	9900	4	8	880	
A4322	Soundaraj.M	18	1	6800	4	60	4080	4	20	1360	
A4336	Ramathal.S	14	1	9800	4	63	6174	4	13	1274	
A4340	Jaithal.K	20	2	9200	4	82	7544	4	19	1748	
A4354	Sudha	22	2	9900	4	61	6039	4	21	2079	
A4396	Saraswathy	30	3	11000	4	85	9350	4	9	990	
A4402	Mary	34	3	6800	4	84	5712	4	12	816	
A4405	Saraswathi	36	3	9800	4	72	7056	4	17	1666	
A4407	Parameswari.K	34.2	3	8900	4	74	6586	4	11	979	
A4447	Indra.R	42	4	1200	2	50	600	2	39	468	
A4450	Indhirani. P	29	2	800	1	88	704	2	12	96	
A4472	Krishnaveni.M	22	2	6800	4	90	6120	4	8	544	
A4477	Geetha.R	24	2	9200	4	78	7176	4	12	1104	
A4539	Gomathy.K	43	4	9900	4	61	6039	4	37	3663	
A4585	Raj. D	47	4	11000	4	53	5830	4	26	2860	
A4591	Vimalraj.P	32	3	6800	4	60	4080	4	10	680	
A4638	Radha	38	3	9800	4	82	8036	4	9	882	
A4648	Prithivi.M	41	4	10800	4	58	6264	4	36	3888	
A4651	Vijayalakshmi. R	26	2	7500	4	60	4500	4	22	1650	
A4671	Chithra.C	30	3	9800	4	67	6566	4	19	1862	
A4675	Balasubramaniam	26	2	6800	4	59	4012	4	13	884	
A4704	Radhika.A	43	4	6900	4	64	4416	4	15	1035	
A4726	Kousalya.N	44	4	1500	2	86	1290	3	13	195	
A4829	Sathishkumar.C	38	3	8800	4	76	6688	4	10	880	
A4849	Chinnasamy. A	28	2	6400	4	60	3840	4	20	1280	
A4868	Kadeswari	28	2	8400	4	88	7392	4	9	756	
A4872	Rajendran.P	30	3	9990	4	89	8891.1	4	8	799.2	
A5148	BEENA.J	30	3	6900	4	80	5520	4	14	966	
		0	1	0	1		0	1		0	
		20	2	1000	2		500	2		500	
		30	3	2000	3		1000	3		1000	
		40	4	4000	4		1500	4		1500	

ART ID	NAME	RBC COUNT	RBC Count Cat	PATELET COUN	Platelet Cat	time	Time Cat	MCV	MCv Cat	MCH	
A2894	Lalitha	1.9	1	0.4	1	30	1	74	2	26	
A2933	K.Prakash	1.6	1	0.2	1	40	1	72	2	24	
A3137	Rabin Raj	1.7	1	0.5	2	45	1	68	1	23	
A3186	Siddhik	2	2	2.6	4	60	2	60	1	22	
A3225	Venkadapathy	2.2	2	2.9	4	73	2	58	1	24	
A3329	Nagarani .R	1.9	1	4	4	78	2	98	4	24	
A3424	Ranjith kumar	2.3	2	3.5	4	74	2	107	5	24	
A3439	Latha.B	2	2	5.6	4	80	2	80	3	28	
A3527	Gunasekaran.M	1.8	1	2.4	4	90	3	56	1	25	
A3547	Muralidharan.N	1.9	1	1.9	4	30	1	62	1	26	
A3590	Devi	2.1	2	0.1	1	32	1	80	3	24	
A3866	Duraisamy	2.1	2	3.6	4	46	1	108	5	25	
A3906	Selvaraj	1.8	1	2.4	4	78	2	60	1	25	
A4044	Arumugam,R	1.4	1	3.5	4	90	3	54	1	24	
A4069	Suresh	1.5	1	3.6	4	92	3	98	4	26	
A4108	Aruchasamy	1.9	1	2.9	4	80	2	62	1	22	
A4120	Chitra.A	1.8	1	2.9	4	24	1	80	3	24	
A4144	Shanmugam	1.6	1	0.6	2	92	3	64	1	24	
A4148	Krishnakumar.k.r	2.4	2	1	3	108	3	78	2	24	
A4152	Ashokan	2.2	2	3.8	4	90	3	88	3	26	
A4153	Sheeba	2.7	2	2.9	4	80	2	86	3	28	
A4163	Jegathambal	2.3	2	3	4	95	3	102	5	22	
A4196	Ponnusamy. N	2.7	2	4	4	80	2	100	5	26	
A4200	Thangamani	1.9	1	3.2	4	120	4	90	4	22	
A4278	P.Selvi	3.2	3	1.7	4	49	1	92	4	28	
A4322	Soundaraj.M	3.4	3	2.7	4	30	1	60	1	22	
A4336	Ramathal.S	1.8	1	6.2	4	62	2	88	3	22	
A4340	Jaithal.K	2	2	3.5	4	68	2	98	4	26	
A4354	Sudha	2.6	2	1.8	4	82	2	101	5	25	
A4396	Saraswathy	2.3	2	0.4	1	20	1	88	3	28	
A4402	Mary	3.4	3	0.2	1	60	2	89	3	31	
A4405	Saraswathi	3.1	3	0.5	2	86	2	92	4	29	
A4407	Parameswari.K	2.9	2	0.45	1	48	1	94	4	33	
A4447	Indra.R	3	3	0.6	2	60	2	78	2	25	
A4450	Indhirani. P	4.2	4	0.1	1	78	2	82	3	32	
A4472	Krishnaveni.M	1.9	1	1.6	4	84	2	111	5	24	
A4477	Geetha.R	1.8	1	4	4	88	2	80	3	24	
A4539	Gomathy.K	2.3	2	0.04	1	68	2	83	3	31	
A4585	Raj. D	3.6	3	0.19	1	60	2	80	3	29	
A4591	Vimalraj.P	1.8	1	0.88	2	92	3	77	2	24	
A4638	Radha	2	2	0.45	1	90	3	76	2	26	
A4648	Prithivi.M	3.6	3	0.48	1	40	1	79	2	25	
A4651	Vijayalakshmi. R	1.9	1	2.9	4	43	1	70	2	22	
A4671	Chithra.C	4.3	4	0.75	2	60	2	99	4	22	
A4675	Balasubramaniam	1.8	1	2.4	4	62	2	58	1	20	
A4704	Radhika.A	3.8	3	0.92	2	66	2	106	5	24	
A4726	Kousalya.N	3.4	3	0.6	2	43	1	102	5	26	
A4829	Sathishkumar.C	2.2	2	0.35	1	49	1	88	3	35	
A4849	Chinnasamy. A	1.5	1	2.3	4	90	3	52	1	18	
A4868	Kadeswari	2.2	2	1.7	4	88	2	78	2	24	
A4872	Rajendran.P	2.8	2	2.5	4	58	1	86	3	24	
A5148	BEENA.J	2.6	2	0.88	2	34	1	95	4	34	
		0	1	0	1	0	1	0	1	0	
		2	2	0.5	2	60	2	70	2	25	
		3	3	1	3	90	3	80	3	30	
		4	4	1.5	4	120	4	90	4		
								100	5		

ART ID	NAME	MCH Cat	type of anemia	ART REGIMEN	MCV value afte	Hb 2nd	Hb Inc	Hb Inc %	Hb Inc % Cat	Hct 2nd	Hct Inc
A2894	Lalitha	2	2	ZLN	98	10.2	4.2	70	2	28	6
A2933	K.Prakash	1	2	ZLN	93	9	4	80	2	29	11
A3137	Rabin Raj	1	2	ZLN	81	10	2	25	1	30	16
A3186	Siddhik	1	2	ZLN	84	9	3	50	2	32	9
A3225	Venkadapathy	1	2	ZLN	81	11	3	37.5	1	26	2
A3329	Nagarani .R	1	4	ZLN	80	9	4	80	2	28	12
A3424	Ranjith kumar	1	4	ZLE	110	10	3	42.85714286	1	32	14
A3439	Latha.B	2	1	ZLN	102	9.8	1.8	22.5	1	40	14
A3527	Gunasekaran.M	2	2	ZLN	92	9.8	5.8	145	3	42	20
A3547	Muralidharan.N	2	2	ZLN	84	11	3	37.5	1	29	11
A3590	Devi	1	3	ZLN	104	10	4	66.66666667	2	40	19
A3866	Duraisamy	2	4	ZLN	106	9.9	2.9	41.42857143	1	25	8
A3906	Selvaraj	2	2	ZLN	96	9.8	3.8	63.33333333	2	24	4
A4044	Arumugam,R	1	2	ZLN	92	9.2	6.2	206.6666667	4	30	16
A4069	Suresh	2	4	ZLE	100	9.1	4.1	82	2	26	10
A4108	Aruchasamy	1	2	ZLN	90	8.9	4.9	122.5	3	24	8
A4120	Chitra.A	1	3	ZLN	89	10.4	4.4	73.33333333	2	23	4
A4144	Shanmugam	1	2	ZLN	102	10.4	6.4	160	4	26	10
A4148	Krishnakumar.k.r	1	2	ZLN	110	11	7	175	4	29	13
A4152	Ashokan	2	3	ZLN	99	10.2	5.2	104	3	32	12
A4153	Sheeba	2	3	ZLN	102	9.8	3.8	63.33333333	2	31	13
A4163	Jegathambal	1	4	ZLN	103	11	3	37.5	1	26	2
A4196	Ponnusamy. N	2	4	ZLE	102	10	8	400	4	36	20
A4200	Thangamani	1	3	ZLN	118	10	6	150	4	34	18
A4278	P.Selvi	2	3	ZLN	91	11	5	83.33333333	2	28	12
A4322	Soundaraj.M	1	2	ZLE	90	11.4	5.4	90	2	29	11
A4336	Ramathal.S	1	3	ZLN	89	11.1	7.1	177.5	4	34	20
A4340	Jaithal.K	2	4	ZLE	96	10.2	5.2	104	3	37	17
A4354	Sudha	2	4	ZLN	106	10.1	3.1	44.28571429	1	32	10
A4396	Saraswathy	2	3	ZLN	98	12	1.9	18.81188119	1	36	6
A4402	Mary	3	1	ZLN	94	13.2	3.2	32	1	38	4
A4405	Saraswathi	2	1	ZLN	90	12.3	3.2	35.16483516	1	43	7
A4407	Parameswari.K	3	1	ZLN	94	13.2	3.9	41.93548387	1	42	7.8
A4447	Indra.R	2	2	ZLN	92	1.5	-7.9	-84.04255319	1	43	1
A4450	Indhirani. P	3	1	ZLN	88	10.8	0.5	4.854368932	1	34	5
A4472	Krishnaveni.M	1	4	ZLN	102	10.3	2.3	28.75	1	37	15
A4477	Geetha.R	1	3	ZLN	84	10.1	4.1	68.33333333	2	34	10
A4539	Gomathy.K	3	1	ZLN	94	11.9	3.4	40	1	42	-1
A4585	Raj. D	2	1	ZLN	98	11	0.6	5.769230769	1	45	-2
A4591	Vimalraj.P	1	2	ZLN	102	11.4	2.4	26.66666667	1	33	1
A4638	Radha	2	2	ZLN	100	12	2.6	27.65957447	1	36	-2
A4648	Prithivi.M	2	2	ZLN	96	13.4	4.1	44.08602151	1	45	4
A4651	Vijayalakshmi. R	1	2	ZLN	88	13.4	6.4	91.42857143	2	43	17
A4671	Chithra.C	1	4	ZLN	114	13	3.2	32.65306122	1	26	-4
A4675	Balasubramaniam	1	2	ZLN	82	9.3	4.3	86	2	28	2
A4704	Radhika.A	1	4	ZLN	108	11.3	2.4	26.96629213	1	42	-1
A4726	Kousalya.N	2	4	ZLN	106	9.5	-0.3	-3.06122449	1	39	-5
A4829	Sathishkumar.C	3	1	ZLN	90	10	1.4	16.27906977	1	41	3
A4849	Chinnasamy. A	1	2	ZLN	78	11	3	37.5	1	33	5
A4868	Kadeswari	1	2	ZLN	89	10.2	4.2	70	2	35	7
A4872	Rajendran.P	1	3	ZLN	88	11.3	3.3	41.25	1	38	8
A5148	BEENA.J	3	1	ZLN	98	10.7	0.6	5.940594059	1	41	11

								67.88814042			
		1						0	1		
		2						50	2		
		3						100	3		
								150	4		

ART ID	NAME	Hct Inc %	Hct Inc% Cat	platelet 2nd	Platelet Inc	Platelet Inc %	Platelet Inc % C	TC 2nd	Tc Inc	TC Inc %	TC Inc% Cat
A2894	Lalitha	27.27272727	1	1.4	1	250	4	6900	5400	360	4
A2933	K.Prakash	61.11111111	2	2.4	2.2	1100	4	7800	7000	875	4
A3137	Rabin Raj	114.2857143	3	1.5	1	200	4	6700	5500	458.3333333	4
A3186	Siddhik	39.13043478	1	2.7	0.1	3.846153846	1	9600	2800	41.17647059	1
A3225	Venkadapathy	8.333333333	1	2.9	0	0	1	9800	600	6.52173913	1
A3329	Nagarani .R	75	2	3.8	-0.2	-5	1	6700	900	15.51724138	1
A3424	Ranjith kumar	77.77777778	2	5.6	2.1	60	2	9800	-2100	-17.64705882	1
A3439	Latha.B	53.84615385	2	4.5	-1.1	-19.64285714	1	10000	-900	-8.256880734	1
A3527	Gunasekaran.M	90.90909091	2	4.7	2.3	95.83333333	2	11000	1200	12.24489796	1
A3547	Muralidharan.N	61.11111111	2	3.6	1.7	89.47368421	2	13000	2100	19.26605505	1
A3590	Devi	90.47619048	2	1.6	1.5	1500	4	5600	4300	330.7692308	4
A3866	Duraisamy	47.05882353	1	4.5	0.9	25	1	6900	200	2.985074627	1
A3906	Selvaraj	20	1	3.6	1.2	50	2	10900	-1100	-9.166666667	1
A4044	Arumugam,R	114.2857143	3	4.2	0.7	20	1	11000	1100	11.11111111	1
A4069	Suresh	62.5	2	2.3	-1.3	-36.11111111	1	9000	-800	-8.163265306	1
A4108	Aruchasamy	50	2	3.5	0.6	20.68965517	1	13000	2000	18.18181818	1
A4120	Chitra.A	21.05263158	1	4.3	1.4	48.27586207	1	6700	-100	-1.470588235	1
A4144	Shanmugam	62.5	2	2.1	1.5	250	4	4500	2800	164.7058824	4
A4148	Krishnakumar.k.r	81.25	2	1.9	0.9	90	2	6900	-1900	-21.59090909	1
A4152	Ashokan	60	2	3.9	0.1	2.631578947	1	9200	-600	-6.12244898	1
A4153	Sheeba	72.22222222	2	4.5	1.6	55.17241379	2	10500	-500	-4.545454545	1
A4163	Jegathambal	8.333333333	1	5.6	2.6	86.66666667	2	7800	1000	14.70588235	1
A4196	Ponnusamy. N	125	3	5.9	1.9	47.5	1	8800	-400	-4.347826087	1
A4200	Thangamani	112.5	3	4.5	1.3	40.625	1	7800	-2100	-21.21212121	1
A4278	P.Selvi	75	2	3.2	1.5	88.23529412	2	6500	-4500	-40.90909091	1
A4322	Soundaraj.M	61.11111111	2	4	1.3	48.14814815	1	7800	1000	14.70588235	1
A4336	Ramathal.S	142.8571429	3	5.6	-0.6	-9.677419355	1	8900	-900	-9.183673469	1
A4340	Jaithal.K	85	2	5	1.5	42.85714286	1	9900	700	7.608695652	1
A4354	Sudha	45.45454545	1	2.3	0.5	27.77777778	1	9000	-900	-9.090909091	1
A4396	Saraswathy	20	1	4.5	4.1	1025	4	9800	-1200	-10.90909091	1
A4402	Mary	11.76470588	1	3.7	3.5	1750	4	7800	1000	14.70588235	1
A4405	Saraswathi	19.44444444	1	1.8	1.3	260	4	6800	-3000	-30.6122449	1
A4407	Parameswari.K	22.80701754	1	1.6	1.15	255.5555556	4	5900	-3000	-33.70786517	1
A4447	Indra.R	2.380952381	1	2.1	1.5	250	4	4900	3700	308.3333333	4
A4450	Indhirani. P	17.24137931	1	1.2	1.1	1100	4	3600	2800	350	4
A4472	Krishnaveni.M	68.18181818	2	1.8	0.2	12.5	1	6700	-100	-1.470588235	1
A4477	Geetha.R	41.66666667	1	2.9	-1.1	-27.5	1	8700	-500	-5.434782609	1
A4539	Gomathy.K	-2.325581395	1	1.1	1.06	2650	4	4700	-5200	-52.52525253	1
A4585	Raj. D	-4.255319149	1	1.9	1.71	900	4	6900	-4100	-37.27272727	1
A4591	Vimalraj.P	3.125	1	2.3	1.42	161.3636364	4	7800	1000	14.70588235	1
A4638	Radha	-5.263157895	1	3.4	2.95	655.5555556	4	6000	-3800	-38.7755102	1
A4648	Prithivi.M	9.756097561	1	2.6	2.12	441.6666667	4	8000	-2800	-25.92592593	1
A4651	Vijayalakshmi. R	65.38461538	2	3.6	0.7	24.13793103	1	9000	1500	20	1
A4671	Chithra.C	-13.33333333	1	2.9	2.15	286.6666667	4	5800	-4000	-40.81632653	1
A4675	Balasubramaniam	7.692307692	1	3.6	1.2	50	2	7800	1000	14.70588235	1
A4704	Radhika.A	-2.325581395	1	2.6	1.68	182.6086957	4	2700	-4200	-60.86956522	1
A4726	Kousalya.N	-11.36363636	1	2.3	1.7	283.3333333	4	1200	-300	-20	1
A4829	Sathishkumar.C	7.894736842	1	2	1.65	471.4285714	4	3400	-5400	-61.36363636	1
A4849	Chinnasamy. A	17.85714286	1	3.5	1.2	52.17391304	2	5400	-1000	-15.625	1
A4868	Kadeswari	25	1	1.9	0.2	11.76470588	1	5600	-2800	-33.33333333	1
A4872	Rajendran.P	26.66666667	1	2.5	0	0	1	6700	-3290	-32.93293293	1
A5148	BEENA.J	36.66666667	1	2.9	2.02	229.5454545	4	9000	2100	30.43478261	1
		44.42389957				292.2711925				46.96995005	
		0	1			0	1			0	1
		50	2			50	2			50	2
		100	3			100	3			100	3
		150	4			150	4			150	4